

Club of Rome comes in from cold?

A small club of well-intentioned people, best known for its past warnings of global catastrophe, has now produced a more persuasive vision of apocalypse and its avoidance.

Is the world about to end, and if so, how and when? This apocalyptic question is most often asked from church pulpits. The Club of Rome first burst on public attention in 1972 by asking (and answering) the question in book form, with the publication of *The Limits to Growth* by Denis H. Meadows and his wife Donella H. Meadows. That exercise, based on a computer model of some aspects of the physical Earth, commanded more attention than respect: the modelling may have been sophisticated for the time, but the modellers had no way of telling how then current trends in energy consumption, pollution and population growth would be modified by each other — and by the passage of time. The Meadowses' vision of apocalypse did not compel conviction, putting the Club of Rome under a cloud for twenty years. But now, against first appearances, Alexander King and Bertrand Schneider have done a better job in *The First Global Revolution* (Simon & Schuster, London and New York).

Why should that be a surprise? Octogenarian King, after all, has had several distinguished careers as, for example, a British civil servant and as head of the scientific section of OECD in the 1960s when it was the Organization for European Cooperation and Development. (In the modern acronym, 'E' stands for 'economic'.) And Schneider has had a distinguished career as a French diplomat. The difficulty is partly stylistic; the authors specialize in apocalyptic writing of the kind in which sentences, each full of foreboding, are concatenated as if from a pulpit of fundamentalist denomination. Then, economic growth is mostly "headlong", environmental degradation "continuing" and waste (of resources or military spending) "criminal". One result is that the argument is hidden from the reader by the richness of the images in which the text abounds. The message is not clarified by the use of the made-up French-sounding word *problematique* to describe the human dilemma and (an innovation) of *resolutique* for its resolution.

The merits of the argument are its differences from that of *The Limits to Growth*. First, King and Schneider's forecasts of the future do not pretend to spurious arithmetical precision. On population growth, for example, they are content to acknowledge the estimated fall of female fertility in developing countries and to use a simple qualitative sentence to conclude that their populations are still certain to increase substantially. The argument is sufficient for their purpose and, in any case, is all that the

facts will bear. To be sure, King and Schneider fall into a few familiar traps, as when they claim it to be absurd that modern agriculture may use 9,000 kcal of inputs to produce 2,000 kcal of food, forgetting that people cannot yet eat fertilizer or the fuel from which it may be made. But they break most decisively from the Meadowses in their acknowledgement that the physical portents of apocalypse are not the only ones that matter. So, for example, do the management of the rich economies of the world, the avoidance of warfare and the ways in which countries of all kinds are governed. Environmentalists everywhere should take note.

And nobody should complain that, having wound up their audience to a sense of doom which, they insist, is avoidable, King and Schneider should have little that is crisp to offer by way of remedy. They ask for an abolition of the arms trade (on the grounds that it is inherently dangerous and a waste of resources), they plead (as everybody must) that the importance of global warming should be generally acknowledged and agreements reached on its abatement (but it is improbable that much good will be done even if "world leaders ... express strongly their conviction" in the need for "a worldwide campaign for energy conservation and efficiency") and they ask that the pattern of development in developing countries should be, with the agreement of all concerned, redirected towards of the advancement of the rural economies of the poor countries. The avoidance of apocalypse, it seems, can after all be rational and tidy. Only towards the end is the predictable echo of what would have been heard from the religious pulpits, when King and Schneider plead that egotism and greed should be made to vanish. No wonder that King and Schneider proclaim themselves to be optimists.

Will it be that simple? The merits of what King and Schneider have done for the Club of Rome is that of putting global environmental problems in the wider context they deserve. But there are many points at which they pull their punches. Thus, while readily upbraiding the governments of industrialised states for the mismanagement of their economies and their spending on weapons of various kinds, they rarely note that mismanagement by the governments of developing countries is not merely a common reason why development assistance fails to achieve results, but also of continued population growth. Their optimism also gets the better of them in the sense



that they imply that good sense and the application of sufficient resources will somehow make the development problem come right. It seems at least as likely that some of the developing countries will find themselves incorrigibly beyond the pale of the development assistance richer governments are politically able to provide. That is a dreadful prospect, but no less real on that account.

Even King and Schneider would be more convincing if they were more explicit about the accomplishment of their worthy goals. It is right to say, for example, that the management of a world consisting of a host of small and nationalistic nations will complicate the management of the global environment, but insufficient then to discuss the difficulties of making democratic systems work. There are one or two dark hints, perhaps unintended, that everything would be simpler if stability were weighed together with democracy as social goals — and if the media were somehow made more responsible. The trouble is that if apocalypse is a present danger, it will have to be avoided in the real and imperfect world. King and Schneider, like the Club of Rome itself, exude something of the belief that, if only they were trusted with putting things to rights, they would manage well enough. But that has become an unpopular way of solving even important problems. And they are unlikely to be given the chance. □

AIDS talk goes on

Next year's international gathering on AIDS is to continue, but to what end?

THE eighth international AIDS conference, which has been in doubt since its Harvard University organizers refused to hold it at Boston in protest at US immigration policy barring HIV carriers from the United States, is back on track. Max Essex and Jonathan Mann announced on 3 September that the meeting will definitely take place in 1992, either in the Netherlands or Spain. Speaking to some 600 AIDS researchers and activists at Robert Gallo's annual laboratory meeting in Bethesda, Maryland, Mann said that the 'crisis' created by US policy presented an opportunity to reassess the AIDS conference, which attracts approximately 10,000 participants.

The organizers of AIDS 1992 promise a meeting that is more international in focus than past conferences have been. They want to pay more attention to AIDS in developing nations where the epidemic is most serious and less attention to strictly national issues, such as US Food and Drug Administration policy. There is an expectation that the social sciences will also be high on the agenda. These are worthy goals but fail to explain what the conference really accomplishes.

Why, one still asks, would anyone want to hold such a meeting? The following 'objectives' have been put forward. First, the meeting is a forum for new information—a point that can be contested. Second, it is an occasion to foster "literacy about AIDS in the humanistic sense" and, third, the mere sight of 10,000 colleagues in one room

provides a sense of 'solidarity' that carries AIDS researchers through to the next conference. It is not immediately clear that such vague objectives justify the millions of dollars that the conference costs, especially when hundreds of AIDS conferences are held around the world all year long with less fanfare and, quite likely, with more significant exchange of information. □

Dead Sea Scrolls

Using a concordance and a computer, scholars have published an unauthorized edition of some of the scrolls.

BIBLICAL scholars in the United States have published an unauthorized but apparently quite reliable version of one of the Dead Sea Scrolls*, thereby breaking the hold a coterie of authorized researchers and their intellectual descendants have had on the ancient writings since their discovery in 1947 in caves in what is now Israel. Using an official concordance produced in the late 1950s (but not circulated until 1988) and a desk top computer called Rabbi, Ben Zion Wacholder and Martin G. Abegg of Hebrew Union College in Cincinnati, Ohio reconstructed the text of Hebrew and Aramaic writings from what is known as Qumran Cave Four.

The text, which can be easily read only by scholars in the field, includes the calendars with festival, lunar and historical data. For instance, there has been a debate about whether the Essenes, a Jewish sect to which Jesus may have belonged, were celibate. Apparently, they were not.

When the scrolls were first discovered, scattered among 11 caves, the right to publish them was assigned by the government of Jordan, which then controlled the caves, to a small group of scholars who were expected to translate the entire opus within a few years. Forty years later, only an estimated 20 percent of the text has been published. It was this slow pace that drove Wacholder to act.

"Frustrated by the realization that at the current rate of publication we shall all be dead when the corpus of the Dead Sea texts becomes available to the world, we began reconstructing the [concordance] as an experiment," he writes. The concordance lists each document in the corpus, each word in it, and the adjacent words in the text so that passages can be assembled like pieces of a puzzle. Wacholder and Abegg expect to publish at least five more fascicles before they are done.

The fact that a small group of scholars has kept such historically important data secret for so long is appalling. If scientific data were at issue, the research community would (and has) rise up in righteous wrath, demanding access. A similar presumption of scholarly access should apply in the humanities as well, and certainly in this case. Although some of the official scholars have complained in the press that the unauthorized publication amounts to theft of data in the concordance, the new publication is a service to scholarship. □

*A Preliminary Edition of the Unpublished Dead Sea Scrolls. Biblical Archaeological Society, 3000 Connecticut Avenue, NW., Washington, D.C. 20008

NASA delays big missions in space science shakeup

■ Outrage at solar observatory decision

■ Lunar lander may be split up

Washington

At least no one can accuse the National Aeronautics and Space Administration (NASA) of reacting slowly this time. Less than two months after the Senate Appropriation Committee passed a spending bill that restricts NASA space science to 5 per cent growth over the next three years — after a growth of nearly 20 per cent a year for five years — NASA has already halted one major mission and is in the process of redesigning another.

In a 22 August memorandum to the Goddard Space Flight Centre, Lennard Fisk, NASA's associate administrator for space science and applications, ordered all work on the \$750 million Orbiting Solar Laboratory (OSL) to be stopped "as soon as possible". Although the solar laboratory programme had not yet been approved by Congress (it had been scheduled as a 'new start' in the 1994 budget) NASA has already spent \$51 million on its design. Fisk argued that it was better to retain the current design for the solar laboratory in the hope of a better economic climate late in the decade than to slash it to an affordable programme today.

NASA is also considering scrapping the planned lunar lander — an important early component in the current plans for a manned mission to Mars — in favour of smaller and cheaper lunar probes, assistant associate administrator Joseph Alexander said last week. No memorandum has gone out cancelling the lander, however, because little is being spent on it at present. For the second consecutive year, Congress has deleted all funding for the Mars mission. Until Congress decides "to take the initiative seriously", says Alexander, the lunar lander will remain a low priority.

The space science changes come on the advice of the Space Science and Applications Advisory Committee, a NASA advisory panel that had already been scheduled to meet in Woods Hole, Massachusetts, on 29 July, less than two weeks after the Senate vote. Shortly before the meeting, Fisk changed the ground rules to reflect the unexpected new budget restrictions. Rather than set priorities based on the assumption of continued healthy growth in space sciences, Fisk asked the panel to cut back the current 'core programme' until it could be contained within 5 per cent annual growth.

Faced with that challenge, the Woods Hole panel recommended a radical restructuring of the 'queue' of planned space

missions. With top priority given to keeping the space physics disciplines healthy, the panel chose to favour frequent launches of small and relatively cheap platforms that could keep many scientists busy. NASA should complete its 'Great Observatory' quartet, the panel said, by adding the Space Infrared Telescope Facility in 1995 to the three already up or under construction — Hubble Space Telescope, the Advanced X-Ray Astrophysics Facility and the Gamma Ray Observatory. But the solar laboratory — another 'big-ticket' item — would have to wait.

"They found themselves in the situation where they had a project that is of unquestionable merit but is rather expensive," Alexander says. "You can have OSL or a series of smaller missions, about one new start a year starting in 1994. If you're talking about discipline vitality, it's important to get those new starts." Typically it takes about five years for a spacecraft to go from new start to actual launch, he says.

In planetary science, the panel gave its approval for a 'flagship' mission to one of the planets. But unless the budget picture improves, NASA will have to choose between a Pluto flyby and a Neptune orbiter for a programme start in 1996, but it will not be able to begin both.

Solar scientists have reacted with almost uniform outrage to the cancellation of OSL. A letter-writing campaign is already under way to protest both Fisk's decision and the rapid reversal of the Woods Hole panel's priorities.

"We have built facilities, trained students, hired postdoctoral scientists and focused our careers on the kind of science OSL can do, all based on your strong support of OSL," Robert Noyes of Harvard University and Robert Rosner of the University of Chicago wrote to Fisk. "Now with this centrepiece of solar research lost (or at least postponed beyond the horizon), we believe there is significant danger that the solar community will fragment, and all this investment will be lost."

Lockheed Corporation has already set up a \$6 million facility at its Palo Alto Research Laboratory to build an instrument package for the OSL. Three other aerospace companies as well as Germany and Italy have all invested money in the project. "None of these investments would have occurred without the assurance that OSL would be NASA's next new start," wrote David Cauffman, manager of the Lockheed solar and astrophysics laboratory.

"How can the heritage of more than a decade of thoughtful NAS [National Academy of Sciences] and NASA strategic planning be tossed out in a single afternoon?," asked Jack Harvey, a member of a predecessor of the Woods Hole panel, the Space and Earth Science Advisory Committee.

The Woods Hole panel will refine the new space science priorities before submitting final recommendations in November. But as for the solar laboratory, the writing is already on the wall. Despite the protests of the shocked solar physics community, NASA officials have no plans to reconsider their decision to halt the project.

Christopher Anderson

ASTRONOMY

Insult to injury

Washington

THERE must have been a better way to break the news. Ohio State University officials, accompanied by their (American) football team, arrived in Tucson last weekend to tell the University of Arizona that Ohio State was withdrawing from the \$60 million Columbus telescope project atop Mount Graham. The environmental controversy surrounding the endangered Mount Graham red squirrel had nothing to do with it, they said; they had simply been unable to raise enough money to meet their \$15-20 million commitment. And then the Ohio State team, the Buckeyes, trounced the Arizona Wildcats, 38-14.

The announcement came as a surprise to the University of Arizona, which, along with Italy's national observatory, is a one-quarter partner in the project. Arizona officials say they had been discussing the possibility of partnership with four other institutions, of which only the University of Toronto has so far been named. "We were already looking for a fourth partner," says Peter Strittmatter, head of the Arizona astronomy department. "Now we're looking for a third and fourth."

Ohio State's withdrawal may delay telescope construction a year, to a 1994 start. But Strittmatter is not taking the Ohio State decision lying down. "I believe that Ohio State made a legal and moral commitment to the project," he says, adding that legal options are being discussed.

Early this week, C. William Kern, Dean of the Ohio State college of Math and Physical Sciences, resigned his position to protest his university's decision. Ohio State astronomy professor Eugene Capriotti announced his retirement, also in protest.

This is only the latest of the troubles to befall astronomy on Mount Graham. After several years of legal battles with environmentalists who feared that the telescopes would further endanger the red squirrel, Arizona received permission to begin construction of two other telescopes on the site early this year. Christopher Anderson

Research grants or handouts?

Paris

WHEN is a government research subsidy really just an old-fashioned bail-out? Just ask Sir Leon Brittan. The zealous competition commissioner of the European Communities (EC) is on a crusade to level the playing fields of the European marketplace, stamping out unfair government aid to industry wherever he finds it. And woe to the company that falls within his sights, for he has the power to call a research grant a hand-out in disguise — and stop it in its tracks.

Under the Treaty of Rome, the EC's rule book, "any aid granted by a member state in any form which distorts or threatens to distort competition by favouring certain undertakings" is forbidden. Depending on the nature of the funding, that stricture can prohibit research and development subsidies for state-owned companies. And this is something that French officials in particular are finding increasingly restrictive.

This spring, for instance, Brittan halted a French government plan to invest FF 2,650 million (\$450 million) into research and development at Groupe Bull, the French state-owned computer and electronics company. Brittan suspected that the money was really an illegal subsidy to help offset some \$1,200 million in losses last year and perhaps make Bull a more attractive prospect for an outside buyer. Bull defended the aid as essentially no different than what a US electronics company would get from a Pentagon contract, or Japan from its industry ministries. Whatever the truth, Bull will have to do without, at least until Brittan's staff finishes their inquiry this fall.

Since the beginning of the year, Brittan has launched over 15 investigations into suspiciously generous state subsidies, including another FF 4,400 million (\$740 million) of ordinary capital that France wanted to invest in Bull and FF 1,800 million (\$305 million) earmarked to Thomson, the state-owned defence and electronics firm. Indeed, at least four of those 15 cases are in France, something that is at least partly due to the amount of money the country funnels into its industry: an average of 5,700 million ecu (\$4,800 million) a year, third most in Europe after Italy and Germany. But even more galling for Brittan is that France is the continent's least contrite offender.

French officials have accused Brittan of going beyond the intent of the EC rules, and undermining the very reason for state ownership. By demanding that governments act no differently than any other shareholder, they argue, Brittan is keeping France from one of the key advantages of socialism: the ability to make long-term investments in things like research, with-

out demanding that a company show a profit every quarter.

"If we don't support Bull now, it will go bankrupt," says Patrick Baune, US representative of the French state-owned venture capital firm ANVAR. "But in two or three years in the future this kind of support will probably be forbidden."

Special exemptions to the EC rules allow for some government research funding to state-owned companies. But the amount of the support is inversely proportional to its proximity to market. So, for example, EC rules allow 50 per cent state support in basic or 'pre-competitive' research, but only 25 per cent when the research gets closer to development of an actual product.

One way around the muddle is to stick to EC-wide programmes and consortia for

basic research. While Brittan casts a jaundiced eye on state research and development subsidies, Filippo Pandolfi, the EC's research commissioner, is doing everything he can to encourage collaborative state research support. European electronics companies control just 10 per cent of the world market and most — like Phillips of the Netherlands, Germany's Siemens-Nixdorf Information Systems, and Britain's International Computers Ltd. — have posted losses in recent years. Pandolfi is encouraging the companies to form research partnerships and to participate in EC-supported programmes like ESPRIT (information technology), JESSI (semiconductors) and RACE (communications) to leverage their research money. As long as government support is through such international collaborations, Brittan can have little complaint — even if the money ultimately benefits individual companies.

Christopher Anderson

SCIENTIFIC MISCONDUCT

Cold fusion tempest at MIT

Washington

A FORMER Massachusetts Institute of Technology (MIT) official has requested a scientific misconduct inquiry at the university's Plasma Fusion Center, claiming that researchers there manipulated data and that the MIT press office is conducting an "orchestrated attack" against cold fusion and its advocates.

Eugene Mallove, former chief science writer in the MIT news office and author of the recent pro-cold-fusion book *Fire from Ice*, claims in his complaint of 18 August that MIT researchers distorted a data curve so that they appeared to get the same results when their experimental apparatus was loaded with heavy water (containing deuterium) as they did when using ordinary water. He claims that this disguised real differences in heat output between the two experiments, and possible signs of cold fusion.

Robert Parker, head of the fusion centre and an author of the paper (*Journal of Fusion Energy* 9, 133-148; 1990) in which the curves are published, explains that the shifting of the heavy water data came as a result of a computer subtraction designed to compensate for water evaporation. In this case, deuterium cells were initially recorded as generating 20 milliwatts of excess power at certain points in the experiment. But because those measurements are well below the 40 milliwatt uncertainty assigned to the experimental apparatus, the MIT researchers argue that they were not significant.

Mallove also claims that Parker misled him into issuing a press release in May 1989 accusing the Boston *Herald* of misquoting Parker. In the article, Parker is quoted as describing the work of Stanley

Pons and Martin Fleischmann, who claimed to have discovered cold fusion, as a fraud, a statement Parker denied in the press release. Mallove says that he later obtained a tape recording of the interview with the *Herald* which showed that Parker had in fact been quoted accurately in the article and had in fact used the word "fraud" several times. Parker says the usages were not in reference to the disputed work.

In his complaint, Mallove requests an investigation into the handling and representation of the fusion centre data, a decision on whether the *Journal of Fusion Energy* paper should be retracted or amended, and an examination of the behaviour of Parker and MIT researcher Ronald Ballinger "in orchestrating a public attack on the motives of researchers whose work they hoped to prove incorrect".

Robert DiIorio, a spokesman for MIT, declined to comment on the status of Mallove's request. But he says that "no complaint gets dismissed out of hand," and that the usual procedure in such a situation would be to set up a panel to review the allegations and decide whether to start a formal investigation.

In a statement released by MIT, Parker said that the *Journal of Fusion Energy* paper has been reviewed by MIT scientists and that, following the review, "the conclusions of the study stand as published."

Mallove resigned earlier this year from his news office post to protest (according to his resignation letter) the "unfortunate way the [cold fusion controversy] has been dealt with by MIT". He remains a lecturer in science journalism at the university.

Christopher Anderson

'Office ladies' aid research

Tokyo

JAPAN'S rice genome project may be on the verge of having its funding tripled — mainly because of an increased interest in betting on horse races by Japan's 'office ladies'.

That rather curious news was one of the highlights of three meetings in Japan late last month between US and Japanese researchers studying the rice genome. The scientists are attempting to form an international rice genome organization to co-ordinate research among nations, and the sudden prosperity of the Japanese programme makes that country the most likely to take the lead in an international effort.



In April, Japan's rice genome project received official sanction when the Ministry of Agriculture, Forestry and Fisheries agreed to provide \$25 million for a seven-year effort to develop a high-resolution physical map of the rice genome. But the first year's funding of \$2.7 million was not nearly enough to support the 100 scientists that genome project officials had recommended, so project leaders turned to private Japanese companies. This was not completely satisfactory, however, because private companies with an interest in the genome could be expected to restrict access to the results of the research in various ways.

Enter an unlikely saviour: the Japanese Racing Association, which regulates horse racing in Japan. The association returns to racing 75 per cent of the money it earns from wagers but uses the other 25 per cent to support a variety of government projects, including research. And the association finds itself unusually wealthy these days, thanks in great deal to the increased popularity of horse racing among Japan's 'office ladies' — secretaries who have disposable income and spend their money on clothes and entertainment.

The rice genome project has applied to the racing association for \$6 million for the programme's first year of operations, and it now seems likely to receive that amount in October. The money will be funneled through the agriculture ministry to a newly formed Society for Techno-

Innovation for Agriculture, Forestry and Fisheries. This organization, with 160 member companies, will pay the salaries of scientists from Mitsui, Mitsubishi, Japan Tobacco, Sanyo Electric and Kirin Beer who will join the rice genome mapping effort.

This is not the racing association's first genome project. It is also supporting — naturally — a horse genome project, and when the rice genome scientists move into newly built facilities next year, they will share space with researchers working on the horse genome.

The expected additional funding for rice genome research in Japan was the good news at the three meetings — the Third International Workshop on Rice Molecular Biology in Hokkaido, and a forum of the Bio-oriented Technology Research Advancement Institution and the second meeting of the International Rice Genome Organization, both in Tokyo — while the bad news was the failure of US scientists to attract support for their own rice genome project.

The US Department of Agriculture (USDA) is sponsoring an \$11 million Plant Genome Project for 1992, but it has turned down several proposals for major crop genome projects, including one submitted by 10 groups of US scientists for mapping the rice genome. Although the department has not yet formally announced which proposals it will fund, several US scientists at the meetings reported that their own enquiries had found that much of the money will be spent on *Arabidopsis thaliana*, a laboratory model with no direct agricultural value. Jerome Miksche of the USDA's Agricultural Research Service said the department cannot afford to fund individual projects for all the different food crop genomes, and if it funded one, such as rice, then scientists studying every other crop plant would lobby for their own programmes.

Ray Rodriguez, a plant geneticist at the University of California at Davis, argued that it makes sense for the United States to make the rice genome a priority, even though rice is not nearly so important a crop for Americans as for Japanese. Because rice has a relatively small genome — about 450 million base pairs, only one-tenth that of barley and one-fortieth that of wheat — rice can be used as a model system to gain knowledge about the structure and organization of other cereal crops, he said. The genome of *Arabidopsis* is even smaller than that of rice, with only 70 million base pairs, but it is too different from cereal crops to be a valuable model.

Japanese scientists said they would like a US rice genome project to represent US scientists in an international organization that would coordinate the flow of data

among researchers worldwide. Of course, individual laboratories can exchange information informally, and one such international exchange has already occurred. Akira Saitoh, who heads Japan's rice genome mapping efforts at the Department of Molecular Breeding at the National Institute for Agrobiological Resources in Tsukuba, traded genome information with Steve Tanksley at Cornell University. But as more groups work on the rice genome, including researchers in Taiwan, Indonesia and the International Rice Research Institute in the Philippines, a formal international organization would help to avoid duplication.

With Japan's efforts so well organized, most US scientists look to it to lead a formal international organization growing out of the International Rice Genome Organization, which is now just an informal group of two dozen scientists promoting international coordination.

Jane Ferrell

SCIENCE MAGAZINES

Disney rescues *Discover*

Washington

WHEN the editors of the popular US science magazine *Discover* found themselves unexpectedly out of work last month after the magazine's parent company went under, they knew that if they were to be published again it might mean wearing some new hats. They did not know, however, that those new hats would come equipped with mouse ears as standard equipment. Last week, under a Mickey Mouse letterhead, Walt Disney Co. announced it had agreed to purchase the magazine and resume publication beginning in November. *Discover* will join a nature documentary service and the popular Epcot Center technology theme park in Disney's science stable, as the entertainment conglomerate moves to bolster its education efforts.

Editor-in-chief Paul Hoffman says *Discover* will retain most of its original staff, although about two-thirds of the employees will move to Burbank, California, Disney's corporate home. The remainder will stay in New York, where *Discover* was published until it was shut down last month by its previous owners, Family Media Inc. (see *Nature* 352, 559; 15 August 1991).

With a circulation of 1.1 million, *Discover* has turned a modest profit in recent years, albeit at the price of taking virtually anything in the way of advertising, even if it ran counter to the magazine's editorial content. That, Hoffman says, will change with the new ownership. *Discover* will no longer accept cigarette advertising and what he calls "fruity New Age ads". Disney also plans to extend the magazine's name to television specials, a possible children's science magazine, and theme park attractions.

Christopher Anderson

Superconductor consortium approaches test

Washington

NEXT month marks the second birthday of the Consortium for Superconducting Electronics, an ambitious attempt by four leading US research institutions to translate scientific prowess into commercial advantage. Two years ago, the move seemed to be just what the doctor ordered to help the United States compete with Japan in developing applications of high-temperature superconductors. Earlier in 1989, a committee to advise President George Bush on high-temperature superconductors — known as the 'wise men's committee' — had recommended the formation of just such consortia to combat a US tendency to focus on short-term goals and neglect research that is still far from commercialization. Hopes and expectations were high.

But after the initial fanfare accompanying the creation of the consortium, which includes IBM, AT&T Bell Labs, the Massachusetts Institute of Technology (MIT) and MIT's Lincoln Laboratory, it virtually sank from sight. What has it been doing?

The two years have been a quiet success, says Richard Ralston of Lincoln Lab, the principal director of the consortium. "We have met all of our first-year [technical] goals and are on a strong track to meet all the second-year goals." The major frustration has been the lack of a small, aggressive firm in the consortium that would push hard for quick commercialization of products from the collaboration's research, but that is likely to be corrected within the month. Although it is still too early to say just how much the consortium will help US industry compete in the coming superconductor market, its participants say this economic experiment has already offered valuable insight into the right way and the wrong way to run a consortium, and its success or failure is likely to influence greatly the formation of such collaborations in the future.

The consortium is founded on complete openness in research and the "full sharing of intellectual property", Ralston says. The charter of the consortium allows each member full rights to commercialize any products that arise from research done in the consortium; furthermore, members agree to give each other rights to research done before the consortium was formed, although the members can charge royalties for previous work.

"The beauty of this consortium is that we basically got upper management out of the way, and scientists could talk to each other," says Bob Dynes, who served as Bell Labs' director for the consortium

until he moved to the University of California, San Diego at the beginning of this year. "Scientists don't care about these barriers [erected by businesses to protect their own interests]."

Nonetheless, Ralston says, it was tricky at first to get researchers to accept the idea of sharing everything — especially researchers at IBM and Bell Labs, two traditional rivals with corporate cultures that emphasize the protection of property rights. But the consortium "broke the ice" by using MIT and Lincoln Lab as neutral ground and by choosing the initial projects carefully so that researchers saw the value of working together and trading on each

potential. But the two years of running the consortium have produced a wealth of experience that could prove valuable to others contemplating such a move.

The glue that holds the consortium together is the graduate students, Ralston says. University faculty and laboratory scientists visit back and forth, usually for a day at a time, but they generally have commitments that keep them close to home. The graduate students, on the other hand, will spend weeks or months away. One of Kastner's students at MIT, for example, goes to IBM to fabricate his devices and to AT&T Bell Labs for measurements, "so he actually shuttles between the three labs,"

Kastner says. "It's good for the students — people at the labs give them a lot of attention."

It's also good for the consortium. It is often the students who figure out how to use the resources at the different locations, says William Gallagher, IBM's director for the consortium. The 'graduate student glue' is one major advantage over consortia that do not include universities.

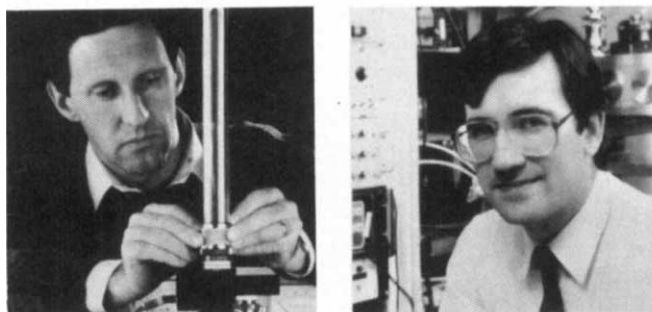
One of the biggest advantages of having different institutions interacting freely on research is the coming together of diverse

research cultures, says Bertram Batlogg, who succeeded Dynes as head of Bell Labs's consortium work. The consortium has allowed the scientists to "learn different ways science and research is done", Batlogg says.

Perhaps the biggest lesson the participants have learned from this collaborative effort concerns something that was initially left out of the mix. With two major industrial labs, a top-notch research university and a government lab, it seemed as if the Consortium for Superconducting Electronics had everything it needed, but experience has proven otherwise.

"What's missing are small commercial companies," Dynes says. The consortium will not have finished its job until its collaborative research has been turned into commercial products, and small firms are the ones that historically have brought cutting-edge technology to the marketplace in the United States. IBM and AT&T are big companies that will not devote a great deal of their attention to new, unproven technologies and that typically take several years to commercialize products, Dynes says, so the consortium really needs "companies whose lives depend on" turning research into results. "That really focuses their attention."

The consortium should fill in this gap in the next few weeks, Ralston says. It has been negotiating with a small superconductor company for about six months, and



Bertram Batlogg of AT&T Bell Labs (left) and William Gallagher of IBM: former competitors, now collaborators.

other's strengths.

The result: "There have been a lot of important technological steps," says Marc Kastner, the MIT director for the consortium, although there has been "nothing really earth-shaking, which is why you haven't heard anything [in the press]." The success story that best illustrates the advantages of the consortium is the development of electronic devices made from a 'trilayer' comprising two layers of niobium, a low-temperature superconductor, sandwiched around a layer of aluminium oxide, an insulator. Lincoln Lab developed the technology for growing the trilayers on a small scale; AT&T grew the trilayers on the large silicon wafers it uses for making electronic circuits; and IBM put the wafers on one of its processing lines, where it etched the trilayers into millions of electronic circuits.

Each of the institutions contributed something the others did not have: Lincoln Lab, a technique for growing the trilayers; AT&T, a facility for working with materials on a large-scale; and IBM, state-of-the-art processing equipment for carving microscopic circuits. The result is that IBM can now turn out made-to-order superconducting circuits on an almost-commercial scale, allowing researchers in the consortium to develop and test new devices.

For now, however, the technological value of the consortium is still mostly

only a few small details remain to be settled.

Somewhat surprisingly, these negotiations have taken as long as the original talks between the four initial members of the consortium. "We've learned that it's easier for two large corporations to agree on something than for a large corporation and a small corporation," Dynes says. The problem, Ralston says, is that "patent rights are more important to small start-ups because they don't have a market yet — rights are a large part of their portfolios. That has made for some rather extensive negotiations."

The lack of a small company has contributed to a second difficulty for the consortium. When it was formed, the consortium was counting on funding of between \$4 million and \$6 million from the Defense Advanced Research Projects Agency (DARPA). But DARPA's money has been coming much more slowly than expected, preventing Lincoln Lab from buying some of the equipment it needs, and the defence agency has so far committed itself to only two years of funding, with an option for a third, while the consortium is planned to last for at least a decade.

One of the things DARPA is waiting for, Ralston says, is to see if the consortium can accommodate small companies. "[DARPA] is not yet convinced the consortium has shown that technical progress can be translated to products, which is what DARPA wants," he says. One question that the agency would like answered is, "How open will the big guys be to letting a small company commercialize the technology?"

In many ways, the Consortium for Superconducting Electronics is likely to serve as a test case for the value of research consortia in general. Ralph Gomory, director of the Sloan Foundation and one of the principal authors of the 'wise men's report on superconductivity' when he was at IBM three years ago, argues that consortia are useful only in certain special cases, mostly in fields that are early in their development where relatively small amounts of money spent intelligently can make a big difference several years on. "High-temperature superconductors offer about as good a case for consortia as you're ever likely to see," he says.

If the Consortium for Superconducting Electronics, with its membership drawn from the cream of US research institutions, does not make a difference to US competitiveness in superconducting applications over the next decade, it will be hard to make the case for other consortia. If, on the other hand, this collaboration proves a success, it could well become a model for others. It is still too early to tell, but with the coming addition of a small company to the consortium, its ability to bring new products to the marketplace will soon be tested.

Robert Pool

Go-ahead for Centocor

Washington

In the highly publicized race to market in the United States the first monoclonal antibody for therapeutic use, Centocor surged ahead of rival biotechnology company Xoma last week, when an advisory committee to the Food and Drug Administration (FDA) recommended that Centocor's new drug for the treatment of Gram-negative bacterial infections and septic shock be approved for marketing in the United States. Xoma was dealt a severe blow when the committee announced that although it would hear Xoma's presentation, it would not make a recommendation at that time. News of the panel's decision sent Centocor's stock climbing and Xoma's plummeting and could give Centocor the edge in the marketplace.

Sepsis, the leading cause of morbidity and death in hospitalized patients, with an incidence of more than 400,000 cases each year in the United States, is most commonly caused by Gram-negative bacteria, such as *Escherichia coli*. Antibiotics, while able to destroy bacteria, have no effect on the endotoxins released into the bloodstream by such Gram-negative bacteria. These endotoxins can lead to septic shock, organ failure and death, often in a matter of hours. Centoxin, Centocor's human monoclonal antibody, binds to endotoxins released by Gram-negative bacteria, neutralizing their effects.

In a placebo-controlled clinical trial, Centocor enrolled 543 patients with sepsis and a suspected diagnosis of Gram-negative bacteraemia. Although Centoxin produced no significant benefit in the treatment group as a whole, use of the

drug among a subset of patients whose blood contained Gram-negative bacteria reduced mortality by 39 per cent. The effect was even more pronounced in patients who were also in septic shock.

Although Centoxin is already approved in the Netherlands, Germany, Luxembourg, Denmark and the United Kingdom, several panel members were less than enthusiastic about the preclinical and clinical data presented by Centocor. The results are "suggestive but marginal", said Donald Summers, a panel member and professor and chairman in the department of cellular, viral and molecular biology at the University of Utah Medical Center.

One of the main criticisms levelled at Centocor was that only patients later confirmed as having Gram-negative bacteria in the bloodstream are likely to benefit from Centoxin. As this diagnosis can be made only after the event, physicians would need to prescribe Centoxin on the basis of a presumptive rather than a definitive diagnosis, which some panel members felt could lead to the treatment of many patients without the Gram-negative bacteria who would derive no benefit.

Unlike Centoxin, Xoma's anti-endotoxin monoclonal antibody, Xomen-E5, is a mouse-derived antibody. In two separate clinical trials, Xomen-E5 had a significant life-saving benefit in a slightly different subset of patients — that is, in those patients with confirmed Gram-negative infections and organ failure but who were not in shock. Xoma was not asked to conduct further trials, but FDA officials said they were unable to make a thorough review of the second study in time for last week's meeting.

Diane Gershon

ENVIRONMENT

Nuclear cleanup costs rise

Washington

THE US Department of Energy (DOE) will need about \$12,000 million more to clean up its nuclear weapons complex for the years 1993 to 1997 than it is likely to receive in funding, according to a DOE report issued last week.

In 1989, the department committed itself to a 30-year programme to clean up all active and inactive sites in the complex, and each year it offers an updated five-year plan. In its new plan, the DOE estimates that it will cost \$40,700 million from 1993 to 1997 to get rid of immediate hazards, to carry out cleanup agreements already made by the DOE and to ensure compliance with environmental regulations.

However, under last year's congressional budget agreement, funding for the DOE cleanup is scheduled to rise by only

10 per cent a year over that period, and in that case the department will receive only \$28,700 million from 1993 to 1997.

How the department will extricate itself from this bind is not clear. Under current budget constraints, Congress would have to pull the extra money from other programmes, and the rising cleanup costs are already putting a strain on DOE's other missions (see, for example, *Nature* **353**, 6; 5 September 1991). Even if it were to receive the additional funds, the DOE has said it may not be able to spend them effectively. On the other hand, if DOE does not spend the money, it may find itself breaking the law with respect to environmental regulations or breaking agreements with the Environmental Protection Agency and various state governments.

Robert Pool

Good news for universities

Tokyo

JAPAN'S Ministry of Education, Science, and Culture (MESC) is at last attempting to infuse some new funds into Japan's rundown university research system. The ministry has requested substantial increases in next year's budget for competitive grants and fellowships to support university research and to reform the engineering and science faculties of Tokyo University, Japan's leading national university. But the new funds are being made available only by squeezing other parts of the ministry's research system.

The ministry's budget request, submitted at the end of last month, is subject to approval by the Ministry of Finance and the Diet and may undergo minor trimming, but substantial changes are unlikely.

Western scientists visiting Japan are often appalled by the rundown state of many of the research laboratories of

annual general maintenance funds for research teams (*koza*) by 25 per cent, or several million dollars, over the next two years. And even more funds will be required in coming years if Tokyo University is to be converted into a 'centre of excellence' as reformers plan.

But to release the extra funds for the universities, the ministry has asked some of its large national research institutes, such as the three laboratories for physiological science, molecular science and basic biology in Okazaki near Nagoya, to carry out their next three-year plan in a five-year period and thereby reduce the annual allocation of funds to these institutes.

The ministry's support for human genome research also seems likely to suffer. Ministry officials say they expect grants for genome research to be about the same as this year, at around ¥400 million (\$3

million), despite earlier hopes of a large increase in funds for the human genome project over the coming years.

Furthermore, MESC is requesting only about ¥100 million for facilities and equipment for a new genome-analysis centre at the medical science institute of Tokyo University. This small request will effectively kill plans by the centre to purchase a supercomputer, at least for the time being. And MESC hopes to increase the number of researchers at the

centre only from four to five. In contrast, the Science and Technology Agency is requesting an increase of more than 50 per cent in funds for human genome research in 1992, to ¥1,520 million (*Nature* 353, 3; 5 September 1991).

Despite the squeeze to help the universities, MESC continues to give strong support to space science and astronomy. About ¥613 million (\$4.5 million) has been allocated for the purchase of giant photomultiplier tubes for what will be the world's largest observatory for neutrinos and proton decay. Plans to build the observatory, called Superkamiokande, in a mine in Gifu Prefecture have been in the works for several years. Funds for excavation of a hole for Superkamiokande are included in this year's budget, but this is the first commitment by MESC to building the detector.

The budget for construction of an 8-metre infrared telescope in Hawaii accounts for most of the rest of the large increase requested for astronomy. And construction of a giant helical fusion device at the new national fusion research institute in Gifu Prefecture takes up much of the increased budget for fusion. But

funds for the huge electron-positron collider TRISTAN continue to fall, with operating time being cut by 200 hours to 2,600 hours next year.

The Institute of Space and Astronautical Science, on the other hand, maintains a healthy budget of more than ¥20,000 million. About ¥100 million in new funds is requested for the institute to prepare for a mission to Mars in 1996 (see *Nature* 353, 8; 5 September 1991).

David Swinbanks

JAPANESE SATELLITES

New testing centre

Tokyo

HAVING failed to exclude foreign suppliers from Japan's lucrative commercial satellite market, the Ministry of Posts and Telecommunications, backed by the government and private companies, plans to establish a satellite testing facility next year to help raise the competitive level of Japan's satellite industry.

The ministry announced last week that it has applied for funds in next fiscal year's budget to set up the new facility, which is expected to cost about ¥12,000 million (\$89 million). The government will provide about one-third of the funds for the centre, while the remaining two-thirds will come from private companies, which are expected to include NEC, Toshiba, Hitachi and Mitsubishi Electric.

Last year, under US pressure, Japan agreed to drop plans by the government's National Space Development Agency to build and launch next-generation communication and broadcast satellites for commercial purposes. Instead, bidding for the supply of such commercial satellites will be open to foreign as well Japanese concerns.

Western (in particular US) companies are strongly placed to win the bids for satellites to be launched in the mid-1990s. And observers say that this is why the government hopes to improve the competitiveness of Japan's industry by establishing the new research centre. D.S.

Sunlight in orbit

Tokyo

SUNLIGHT (*yōkō*) is the name given to Japan's latest X-ray observation satellite, Solar-A, which was launched without a hitch on 30 August by the Institute of Space and Astronautical Science from its Kagoshima space centre in Uchinoura in southern Japan. *Yōkō*, which is orbiting between 520 and 770 km above the Earth, will observe solar flare activity of the Sun during the present solar maximum using hard and soft X-ray telescopes and X-ray spectrometers developed in collaboration with US and UK scientists (*Nature* 352, 96; 11 July 1991). After the equipment has been tested, observations of the Sun are expected to start later this month. D.S.

BUDGET REQUEST FOR MINISTRY OF EDUCATION, SCIENCE AND CULTURE

	thousand million yen	% change from 1991
Grants-in-aid of research	65.1	+ 10.5
Government/industry research	16.8	+ 25.3
Donations from industry	48.1	+ 8.7
Nuclear fusion	10.6	+ 20.7
Accelerator physics (TRISTAN)	14.3	- 9.4
Space science	21.0	+ 1.0
Astronomy	3.2	+ 90.0
Earth science	2.3	+ 1.1
Antarctic research	3.7	+ 20.1

Japan's leading national universities. Calls for a dramatic increase in budget for university research have been increasing in volume in recent years. Akito Arima, president of Tokyo University, has been particularly vocal in his demands for reform.

MESC wants to boost the budget for competitive research grants, which go to university and other MESC researchers, by more than 10 per cent to ¥65,100 million (\$482 million). This is the biggest increase requested in years, but it still runs far short of the doubling in budget that leading academics say is needed.

The ministry hopes to support an extra 300 young scientists and engineers at the universities with postdoctoral and graduate fellowships. And MESC will boost the salaries for next year's 1,400 fellowships by 10 to 23 per cent, all of which will require a 47 per cent increase in the fellowship budget to ¥3,458 million (\$26 million).

The ministry has also applied for extra funds to reform the graduate school of Tokyo University (*Nature* 351, 679; 27 June 1991). Details of the request are not yet available, but the engineering and science faculties are hoping to increase the

Vitamins and intelligence tests

SIR — I write in an effort to counteract the negative impression about the possible value for children of a daily vitamin-mineral supplement given by three articles in a recent issue of *Nature*¹⁻³ discussing a paper by Schoenthaler *et al.*⁴. I was not one of the authors of this paper, but I was mentioned in one of the articles² and I made a statement about the matter for the BBC programme broadcast on 27 February 1991.

I had acted for the Dietary Research Foundation as an adviser and critic of the Schoenthaler study. Some of my points of criticism have been reported in the Blinkhorn article³. It is my opinion that, although the Schoenthaler study is not by itself convincing, it adds to the earlier evidence that supplementary vitamins and minerals improve, for many children, their performance on psychological tests, perhaps by improving their alertness and awareness.

The earlier study that made the greatest impression on me was that of Kubala and Katz⁵. These investigators first measured the amount of vitamin C in the blood plasma of school children in Texas. Half of the children were low in this vitamin, less than 1.10 mg dl⁻¹, and half were high, with more than this amount. They then selected 72 pairs, matched for socio-economic status (family income, education of father and mother), for further study. The average measured IQ of the low vitamin C group was 4.51 units lower than that of the high vitamin C group. After they had received a supplement of 90 mg (one glass of orange juice) per day for six months the tests were repeated: the low group, for which the vitamin C level had risen into the high range, had an average increase in measured IQ of 3.54 IQ units, whereas the average increase for the other group was only 0.02. The study was then repeated with 32 matched pairs, over 18 months with four IQ measurements. The measured IQ increased steadily by 3.6 units as the average plasma vitamin C level increased from 1.03 to 1.55 mg dl⁻¹.

There is also some evidence that an increased intake of nutrients other than vitamin C contributes to this effect⁶⁻⁸.

In the Schoenthaler study, the subjects were not divided into well-nourished and poorly nourished groups, and fewer than half may have been poorly nourished. Most British children are in the poorly-nourished group, as determined by the plasma levels of vitamins. I am confident that the daily intake of a vitamin-mineral supplement would on the average improve their performance in intelligence tests and also improve their general health. The medical authorities have

been astonishingly reluctant to recognize the value of vitamin-mineral dietary supplements, which at low cost can improve every person's health.

One supplement, called Vitachieve, is mentioned in *Nature*². This supplement does not differ much in composition from other vitamin-mineral supplements, and I doubt whether it is more effective than others. My advice is to buy the cheapest supplement, after checking the amounts of the nutrients that you would get for your money. Unless you buy a high-priced product, the money would be well spent.

LINUS PAULING

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Powdered milk

SIR — The issue of Nestlé's marketing of breast milk substitutes in developing countries has been obfuscated by a lack of information about what exactly the offending practice is, and your leading article on the appeal by the Church of England's General Synod for a boycott of Nestlé's coffee in this country (*Nature* **352**, 266; 1991) only clouds the matter further. It misrepresents both the Synod's motives for advocating the boycott and the underlying facts of the matter by parading loose assumption and speculation as fact. It is clear from reports of the Synod debate (*Church Times* 19 July 1991) that the churchmen consider the issue as far from simple, and that all the confounding factors you mention such as the risk of AIDS transfer by breast feeding were taken into consideration before the vote was taken. It is also important to realize that the World Health Organization (WHO) has been condemning Nestlé's activities in this area for years, though sadly to no avail, which is why your assertion that the WHO's demand for the advertising of the benefits of mother's milk "in principle, should ensure that manufacturers will no longer play on the credulity of unsophisticated mothers in developing countries" carries little conviction.

The objection is not simply to over-

selling the benefits of powdered milk formula in poor countries as you state, but to the insidious practice of distributing free milk to maternity wards in order to create a market for the product among nursing mothers by the time they leave hospital, despite the risks inherent in bottle-feeding where clean water is scarce and infection rife. It is this exploitation of the health and lives of the poor for financial gain that is under criticism, and if Nestlé is indeed guilty it is surely appropriate for Synod to register its disapproval by discouraging trade with the offending organization.

It is easy to vilify Nestlé (and the other multinational companies with similar marketing interests in developing countries), in the light of these rumours, but it would be wrong to do so without availing ourselves of the whole story. For similar reasons I think you are unjustified in assuming the action taken by the Church of England's governing body to have been hasty and unthinking. I hope the publicity gained from Synod's decision will at least prompt a long-overdue statement on this issue from Nestlé before too many wild conclusions are drawn from its present reticence.

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Sir — Sadly, the issue raised by "the British Anglican plan to boycott Nestlé coffee" is not as simple as you think. This laboratory is involved in human milk research^{1,2}. Human milk substitutes in any form are less than ideal infant food, but in powdered form the product can be dangerous — and not only because of problems resulting from mixing the powder with poor-quality water and non-sterile conditions. It is difficult for mothers to measure the correct concentration. If there is too little powder, the infant does not get needed nutrients, but if there is too much (and by a narrow margin), there is the real possibility of stressing the infant's metabolic machinery³.

Why has this battle been left to the Anglican church? Where are the regulatory agencies of the industrialized countries? Where are the Food and Drug Administration and the US Department of Agriculture? Women in the United States need to be informed and protected. They are really "the less fortunate counterparts" in this instance.

M. F. GOLDFARB

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Why scientists cover up fraud

SIR — John Maddox (*Nature* 350, 269; 1991) asks why a scientist like David Baltimore should have so vigorously defended what has now proved to be a false position. Philip Weiss raises an even broader issue in his article on scientific fraud (*New York Times Magazine* 29 October 1989), namely why “no one who was equipped to wanted to get to the truth of the matter...”. Although the statement is false, as Weiss reveals, it brings out the essence of a dilemma: why do scientists tend to cover up and deny scientific fraud?

As a scientist saddled with the aftermath of a parallel scandal, the Darsee affair, I wish to suggest a possible answer: researchers routinely accept a certain level of dishonesty and therefore defend larger transgressions that involve the same vice. The particular corruption that I speak of is unearned authorship.

Earned authorship in scientific papers means doing the experiments, analysing the data, working out the theory, writing the paper, reading the literature — these activities enable an author to lecture on the paper in front of a critical audience. Merely encompassing the work under the umbrella of one's interest and ideas does not.

Why do scientists expose themselves in this way? For one thing, putting one's name where it does not belong is rewarded by granting agencies and tenure committees. Close colleagues easily spot perfunctory authors, but more distant evaluators may merely count articles. Separate groups that are funded jointly may also work independently on the project, and a common temptation is to add each other's names to what should be autonomous articles.

But these motives would not explain why senior scientists with numerous grants and important positions would sign their name to an article they have barely read, much less worked on. Perhaps conceit drives unearned authorship, the need to stake a claim to a discovery one is minimally involved with. Intellectual participation is an excuse for this tactic, but that often means having done what any informed colleague would do casually and without credit, except perhaps in the acknowledgements. Because of administrative or monetary relationships between senior and junior scientists, marginal contributions may be elevated to authorship.

At Emory University, during the investigation of the Darsee affair, the argument was made that junior scientists cultivate senior co-authorships in order to get their work published. The review system should not work that way, though

frequently it may. Regardless, this argument puts the blame on the privates and lets off the generals. Established scientists, under pressure to obtain extramural funds, are burdened with the baggage of success: leadership in national societies, membership of editorial boards and grant review panels, travel and lectures, committees and administration. These activities drain the time and the energy of every established investigator, and they make bench research nearly impossible. Yet the pressures to present oneself as being at the vanguard of research are greater than ever.

By accepting or insisting upon unearned authorship, much of the scientific community has forfeited the right to bear witness. Thus, when investigations reveal unbecoming conduct that involves the same crime, scientists close their ranks, because many are guilty of far less spectacular but similar infractions.

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Perspectives for India

SIR — Few would disagree that the developing countries stand to suffer the most from environmental problems such as loss of biological diversity, degradation of ecosystems, environmental pollution and contamination, and other aspects of global change. I suggest that Indian universities have a particular contribution to make by stimulating collaborative ecological training and research to address the regional environmental problems of south Asia.

It is apparent that the traditional university departments designed for compartmentalized teaching of botany or zoology do not provide the kind of training needed for the new generation of ecologists who need to understand complex ecosystems in order to predict, plan and manage the environment and resources of tomorrow. Special emphasis needs to be placed on five areas of research: ecosystems analysis and modelling; conservation and evolutionary ecology; environmental biotechnology; ecology of global change; and ecological economics.

Development of this expertise will require a new university infrastructure, which could take the form of interdisciplinary centres or of wholly new departments such as the one at Jawaharlal Nehru University, New Delhi, but with greater interaction among the disciplines.

Any reorganization within a higher education system will encounter resistance from the inertia of the system and from

worries as to whether environmental concerns will indeed result in an increased demand for ecologists. Advanced teaching programmes will have to be integrated with real life, long-term research programmes so that teaching and research fit snugly with each other. The new approach would further require a coordinated network of sites for monitoring long-term trends, along with year-to-year variability, in selected natural and man-modified ecosystems for facilitating comparative experiments and for studying ecological phenomena that occur on timescales of decades or longer.

I believe that India has a special responsibility for the whole of south Asia because of the country's relatively well-developed scientific infrastructure. Biosphere reserves that have been and are being established in India represent all the ecological conditions of south Asia, from arid desert and wet tropics to alpine regions. Finally, India already has bilateral agreements and exchange programmes with several developed and developing countries, which could be built on in the future.

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Baer facts

SIR — On 28 February 1992, 200 years will have passed since the birth of Karl Ernst von Baer, the distinguished biologist, and at the end of February 1992 the Estonian Academy of Sciences will hold an international conference dedicated to Baer in Tartu, where he studied at the university (1810–14) and spent the last years of his life (1867–76).

In 1976, the Baer Museum was opened in Tartu, where materials connected with Baer are displayed, his letters are studied and investigations in the field of evolutionary theory and the philosophy of biology are carried out. Numerous works by and about Baer, including manuscripts, letters, photographs, pieces of art and other materials, are preserved there. Material on Baer can also be found in Leningrad, Tallinn and Giessen. Only part of this material has been studied and published, including nearly 800 letters by Baer.

One of the main aims of our activities is to publish almost the whole of Baer's collection of letters, and I should be very grateful to anybody who can provide me with information about them. The Baer Museum would be glad to have copies of the letters, or any information on where they are preserved.

VELLO KAAVERE

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Nature's Manifesto for British Science

Everybody has a recipe for revitalizing British science. What follows is one that makes the production of skilled people the centrepiece of action for the next decade (and beyond).

THE next British general election must be held no later than 19 June next year, the fifth anniversary of the last election. Although Britain boasts of having no written constitution, the Parliament Acts dictate the timing. But there is only a small chance that government policies on research — and government attitudes towards research — will be more than peripheral issues at the election. More immediate questions — the performance of the economy and the consequences of British membership of the European Communities (EC), for example, are likely to command much more attention.

One irony in that prospect is that all British political parties acknowledge when asked — or required by circumstances such as an impending election to do so — that policies on and attitudes towards research are vital ingredients of success both in the management of the economy and in charting a course within the EC.

Another is that much of British science is in a mess. The infrastructure (especially in the universities) has been undermined by irrelevant and even malevolent thinking, the pattern of spending has become too rigid, and, now, young people are turning their backs on science. The research enterprise, while still productive, lacks confidence and élan.

All parties agree that science, and the technology it breeds, are the basis of prosperity in the modern world. All of them are also ready with the politically correct nostrums: that the foundations of healthy innovation begin in nursery school, that the transition from secondary to tertiary (university, polytechnic and college) education must be more flexible, and certainly different, that there must be immeasurably more sensitive but as yet unspecified ways of making sure that science is harnessed to the cause of technology — and that private industry (for its own good) should pay a larger share of the total cost.

But none of the British political parties appears much more than merely earnest in its espousal of the cause of science. Yes, research and development are essential ingredients of industrial im-

provement and eventually of prosperity, for which reason policies on research and development must be drawn up and then carried through responsibly. But, in the process, each party gives the impression that the science lobby (of which this journal is adventitiously a part) has become a nuisance that must be placated. Dutifully, each of them (the government Conservative Party, the opposition Labour Party and the Liberal Democratic Party) has published a suitably placatory statement in the past few weeks.

But none of these statements appears to have heeded the logic of the principles it espouses; if science and research, and the technology that they spawn, are the foundation of future prosperity, should not the first goal of a British government be to search for ways of making science and technology stronger, relative both to the present and to the condition of science, research and technology elsewhere? In the event, the maintenance of the status quo seems the most ambitious goal on offer.

The essence of the difficulty is the failure of British politicians, over many decades, to understand the relationship between research, technical innovation and industrial improvement. Their supposition that technical matters are beyond general comprehension (not to mention their own) and their annoyance that such an arcane activity as research should both occasion rows about public spending (as when British governments periodically agonize about continued membership of the European high-energy physics laboratory at Geneva) and the need for frequent bothersome legislation (as on embryo research) has engendered a sense that their applause for the prosperity that research might bring is matched only by their indifference to the true needs of the research community.

It is only fair to acknowledge that the scientific community itself has not always been a trustworthy guide to realism in these affairs. Especially in the two decades after the Second World War, the British scientific community fell into the habit of promising its public sponsors 'world-beating' innovations at virtually no development cost (the world's first

commercial mainframe computer in 1950, the world's first commercial passenger jet at about the same time, three successive generations of nuclear reactors within the succeeding decade, and the world's first thermonuclear reactor just two years later). In the absence of full recantation, who in the scientific community can now blame a British politician for looking for evidence of this year's discovery in next year's gross national product (GNP)?

What follows is both a survey of what has gone wrong in and with British science in the years since the Second World War and a guess at what might even at this stage be done to put things right. It is offered as a contribution to the arguments that will precede the next general election, and in the hope that policies on science and technology will become the electoral issues they deserve to be.

Political parties wishing to use this manifesto as their own may do so without charge or even acknowledgement. But those tempted to settle for some parts of it, but not others, should be warned that the recommendations hang together, and that half-measures may create more problems than they solve. (Party managers will notice that the editorial "we", confined to the paragraphs in bold type that follow, may be taken over unchanged as references to themselves.)

We start from the position that the state of British science, while desperate, is far from hopeless; that there are imaginative if sometimes radical remedies that could quickly restore the spirits of researchers and even satisfy the anxieties of governments about the British GNP; that the amount of public money being spent on British science, which is important (yet substantial), matters less than the way in which it is spent; and that the perennial British search for a better way of administering civil science is an irrelevance. Organizational issues are, of course, important, but in the past half century they have often impeded the still more important process of defining the objectives of it all.

In what follows, terms such as "basic", "applied" and "strategic" research are, as far as possible, avoided. Instead, the

British research enterprise is described in terms of the three administrative sectors of which it operationally consists — the academic sector, the public sector and the industrial sector. Among other things, this classification is best matched with the mechanisms by which governments support research and influence research policy, as well as with a discussion of the changes needed to make them function more effectively.

Briefly, the academic sector is primarily for the education of young people in science and for research, but is also collectively (taking all nations together) the source of new knowledge of the natural world and also of the wellsprings of industrial innovation. The health of the academic research sector is inseparable from that of the institutions in which it is carried out. The British academic sector has been especially hard hit by administrative decisions in the past decade, but the rot can be traced back at least to the early 1970s. A central theme in what follows is that the energies of the British academic sector must be directed more deliberately (and generously) towards education in research.

The public sector has (at the least) responsibility for the direct support of public policy (as in regulatory matters and military defence); public sector research may be found at the municipal, state, national or international levels, and has been shockingly neglected by British governments in recent years, both financially and by incomprehension.

The industrial research sector exists to improve on the products and processes on which individual companies and their customers depend. Theoretically, the aggregate volume of industrial research and development is the outcome of individual companies' decisions on the relevance of research and development to their commercial success.

In the years immediately after the Second World War, organizations such as the Anglo-American Productivity Council had taken to marvelling that British companies paid such scant attention to the deliberate search for innovation. Since then, support for research and development by the armed services (chiefly in electronics and aeronautics) has been the sheet-anchor of British industrial research activity, the chemicals and pharmaceutical industries excepted. Yet still, by common consent, there is too little industrial research and development. And the direct product thereof — prosperity — remains elusive.

In this litany of discontent, the implication that British science is in terminal decline can be (and has been) disputed. (Government ministers are naturally defensive on the subject.) It is therefore only seemly (but also proper) to

acknowledge that some of what is wrong lies in the research community's own perception of its conditions.

Part of the difficulty is historical. Just as Britain has found the abandonment of empire difficult to accept, so it has learned with difficulty that the twentieth is a more competitive century than the nineteenth. Then, the Daltons, Faradays, Maxwells and Thompsons of their time were unchallenged heroes in a scientific community much smaller than that now seeking an understanding of the natural world, and virtually devoid of organization.

The excitement these legends engendered helped to tempt to Britain the two Braggs (from Australia) and Rutherford (from New Zealand via Canada) — and Rutherford almost single-handedly populated the physics departments of Britain with heads in his own image. When research was still largely the product of individuals' imagination, Britain was also lucky in its 1930s immigrants from mainland Europe (with Born, Krebs, Peierls, Perutz and others). At least in the academic sector (but also elsewhere), it has proved to be surprisingly difficult to adjust to the notion that the successful practice of science requires the collaboration of others as well as flair.

Thus the present low ebb in the morale of British research may be, in part, a consequence of a yearning for a simpler past, when historical accidents could allow smallish countries to have a disproportionate influence on the torrent of discovery. And nothing in what follows should be taken to imply that the now-successful researchers in Britain lack the flair of their predecessors.

Moreover, to the extent that the success of academic researchers can be judged by the numbers of academic papers they publish, and by the frequency with which those are cited by others, the British academic sector remains second only to the United States in productivity. All that should be freely acknowledged. The worry, for the academic sector, centres on what lies ahead. The weakness of the link between discovery, industrial innovation and prosperity is a more durable anxiety.

The policies of successive governments over two decades are chiefly responsible for this state of affairs, often for no other reason than their policies have lacked consistency. The treatment of the academic research sector in the past decade has been especially damaging; that is where remedies must most urgently be sought.

A little muck-raking is, regrettably, unavoidable.

In May 1980, in an address to the Parliamentary Committee on Science and

Technology, the then newly elected prime minister, Mrs Margaret Thatcher, announced that what is called the Science Budget (direct support for the five research councils, the Royal Society and, then, the British Museum (Natural History)) would be "protected", both against inflation and her government's energetic efforts to contain and even reduce public spending.

Inevitably, the announcement was greeted with relief in the academic sector, which is the chief beneficiary of the research councils' spending. After a decade of pressure on research funds, it seemed especially welcome. And Thatcher's promise has indeed been kept; the science budget has grown in the intervening decade from just over £500 million to more than £850 million.

But there were other developments with a countervailing influence. At the end of the year in which Thatcher made her promise, Mr Mark Carlisle (then Secretary of State for Education and Science) announced that the recurrent budgets of the universities would be reduced by 13 per cent over the succeeding three academic years, partly by a direct cut of the annual subvention, partly by requiring that overseas students at British universities should pay much higher tuition fees.

At about the same time, it became plain that the Ministry of Agriculture, Fisheries and Food, which had been committed by the Rothschild review of civil science in 1971 to contributing about a third of the budget of the Agricultural Research Council, planned to reduce its contribution to virtually zero (as it then did).

The first of these developments was especially damaging. The Carlisle edict virtually coincided with the much-delayed publication of the Merrison report (named after the late Sir Alec Merrison) on the 'dual-support system' by means of which research in the academic sector (or at least in universities) had traditionally been supported, partly through recurrent university budgets and partly by means of research grants from research councils. Merrison was the first to calculate ('guess' may be a better word) that 30 per cent of university spending could be counted as research support.

The direct cut in university budgets (9 of the 13 per cent) has not been restored in real terms (although the recurrent budget has increased not quite in line with increasing student numbers), even if most universities have been more successful than seemed likely at the outset in recruiting overseas students at the high fees newly decreed by the government. Since then, both in the administration of science and of higher education, it

has been mostly chop and change.

The first response to the Carlisle cuts by the University Grants Committee (replaced three years ago by the Universities Funding Council) was that universities should not seek to earn their way out of trouble by increasing student numbers. A few years later, its successor was inviting universities to do the opposite in an auction for student members that proved to be a conspicuous failure. At the same time, the enforced decline of university budgets prompted the publicly financed early retirement of academics, but was followed by a scheme due to the then Sir Keith Joseph for hiring younger people (called 'young blood').

Government enthusiasm, early in the decade, for information technology and its potential for creating wealth led first to a scheme by which research councils (notably the Science and Engineering Research Council) and the then University Grants Committee were persuaded to devote funds (some £12 million) to the creation of posts and research projects in the field, but this was followed and overtaken by the more ambitious Alvey programme for research and development in information technology which ran over five years, but was abandoned in 1988.

This setback did not take the edge off the rhetoric of the governments of the 1980s, whose chief concern appeared to have been that the academic sector should contribute more directly to the needs of industry and commerce. Its dependants, universities and research councils, have responded with varying degrees of enthusiasm. Industrial research contracts now constitute a substantial part of the income of many universities while many graduate students are trained in research by means of projects mounted jointly between universities and companies.

Yet the habit of snap decision continues. Although Sir Keith (now Lord) Joseph agreed that the Advisory Board for the Research Councils' advice to him should be published in the interests of open government, his successor-but-one, Mr Kenneth Clarke, has decided that last year's advice should be confidential between him and the council.

And only a few months ago, in a discussion paper on vocational training and higher education, the present government announced both that the polytechnic and university systems would be combined (without arrangements for supporting research across the board) and that the Scottish and Welsh systems of higher education would be run separately from that in England; each proposal will affect the academic sector of the research enterprise in important ways, and would ordinarily have been considered to require

time to mature. In neither case has there been a serious attempt to foretell the consequences (which may nevertheless turn out to be beneficial).

The baleful and personal influence of Mrs Margaret Thatcher cannot be overlooked. On the strength of her first degree in chemistry, her governments resisted many proposed changes of organization by saying that she would take a personal interest in science. But her interests were fickle and narrow-sighted. Her style was to seize on an issue and make a fuss about it. Thus, long after the event, she berated the Medical Research Council for not patenting monoclonal antibodies. Often she would appear to encourage projects and then demand independent reviews of them, like a cat with a mouse.

It is essential that governments should forswear hasty and ill-considered decision-making touching science and research. Snap decisions distract researchers from what they do best, interrupt careers in random ways and (when made apparently on whim) demean the advisory process which is, at present, the only means at the government's disposal for winning the research community's consent to its decisions.

The implications of the proposed changes in higher education must profoundly influence the functioning of the British research enterprise. The number of institutions called universities in Britain will have been increased by nearly a half (to more than 70, depending on definition and the constitution of the University of London). But they will not be equal. Some are already strong in research, others (in science and technology) have only a marginal presence. And many of these institutions are only small, with between 2,000 and 3,000 undergraduate students.

Such a pattern of higher education cannot be stable, let alone economical. Many institutions will find it prudent to concentrate (as the financial pressures from the funding councils already persuade them) on the activities in which they can most easily recruit students, perhaps in business studies or public administration.

In principle, polytechnics (as they still are) have equal rights in applying to the research councils for research grants, but successful applications are only few. Part of the explanation is that teaching loads in polytechnics are so much greater than in universities that their academics have little time for research. Another is that their capacity to promise that the research grants for which they apply would be spent in the 'well-found' laboratories on which the research councils insist is diminished.

The second impediment may be partly removed by the agreement that £125 million will be transferred from the recurrent budget of the Universities Funding Council to the research councils, allowing them to pay the 'overhead costs' of research projects, but the first impediment will remain.

These changes will coincide with a profound change in the pattern of the secondary education of recruits to higher education. All parties appear to agree that the education of school-leavers should no longer be a dedicated preparation for a specialist university course, but that it should be broadened. In principle, the universities agree, despite misgivings in science departments that new entrants will in future be less well-prepared. The danger in this prospect is that the recruitment of young people to science and technology courses in British higher education, already in relative decline, will be further diminished.

For that reason as well as to make the recruitment of potential scientists and technologists more sensitive to young people's conception of the attractiveness of careers in science and technology, the present link between school preparation and higher education should be broken — ideally, completely (as it almost is in the United States). Thus recruitment to science and technology courses should not depend on specialist qualification at the school-leaving stage. A corollary is that science and technology courses should ordinarily take four years, not the now-common three.

During the past few months, with the recognition that important changes are indeed afoot, there has been much talk about alternative patterns for undergraduate courses. Some favour two-year courses for the many students and four-year courses for others. Others advocate a '3 + 1' pattern, with a 'general' degree for the early leavers and an 'honours degree' for those who stay on.

The first pattern seems to reflect the British predilection for mutual merit assessment. Moreover, when there is already good reason to believe that three-year degree courses on the present pattern do not adequately prepare people for careers as free-standing scientists, it is difficult to believe that less intensively prepared students would gain much that is worthwhile from two years of higher education. (There might be a stronger case for two-year courses as preparation for more specialized study — say mathematics followed by computer science — but that is a different argument, as is the case for building into British undergraduate education points at which young people can transfer from one institution to another.) Much the same objection

applies to the '3 + 1' pattern, but less strongly.

It is curious that academics are among the first to say that the extra costs of four-year courses make that option unattainable. Although the governments of the 1980s often behaved as if they would prefer to shrink the proportion of the population in higher education, both the present government and the opposition Labour Party are united in the beliefs that secondary education should be broader and that the rate of participation in higher education should increase. The case for asking them to face the logic of their good intentions is overwhelming.

If Britain is to rebuild its technically qualified workforce, the four-year first degree must be the rule, not the exception.

The relationship between research and undergraduate teaching is also likely to be a contentious issue in the months ahead, especially for what are now the polytechnics. Common experience shows it to be false that all good teachers must also be active and successful researchers. Typically, good departments include a sprinkling of people whose interests lie in teaching rather than research.

But a successful teaching department should also give its undergraduates a first-hand glimpse of the frontiers of its subject, while there are great benefits for undergraduates in their exposure to teachers with varied interests, styles and even temperaments. Yet many of the science and technology departments of Britain's emerging system of higher education are too small for these purposes, let alone to accommodate effective research groups in novel fields.

The goal should be that no substantial department teaching science and technology to undergraduates should be innocent of research. Thus in the new regime, the combined funding council should have the resources for tackling two important structural problems: (1) to enable institutions to grow in size, preferably by mergers, but otherwise by favouring successful institutions at the expense of others; and (2) to encourage the growth of a substantial research presence at institutions at which it appears initially to be absent.

One of the reasons why research should be as widely spread as possible in the emerging system of higher education is that it will widen the net for the recruitment of young people into research. And that is important because Britain needs many more of them. Why? What kinds of people should they be, how should they be chosen and supported and how many of them should there be?

The crucial consideration is qualitative. Even the best undergraduate courses in science and technology only inadequately confront young people with problems that typify what real science is like — how to reply to a question whose answer is unknown. Yet that is the kind of question repeatedly thrown up in the real world. (When the study of engineering was simpler than it has become, engineering departments were consistently more able than science departments at problem-solving, but now there is so much more to learn.) This leads quickly to the conclusion that attempts to augment Britain's capacity for innovation must begin with a consideration of the mechanisms for education in research.

Several questions arise. Over many decades, politicians of all political persuasions have been wringing their hands with regret that a research community capable of winning Nobel prizes should be almost as conspicuous for its lack of successful industrial innovation. "We", the argument goes, "support research. Why does not research support the national economy?" The complaint leads to the demand that research should be more 'relevant'.

Those who make it overlook, or have never understood, the huge gap there must be between discoveries about the natural world, which are the chief (but not exclusive) concern of the academic sector of the research community, and the development of innovations that can succeed on world markets.

Yet a whole quarter of a century passed between the discovery (in Britain) of the structure of DNA and the founding of the first flush of companies (mostly in the United States) intended to make money from it. And the conclusive proof that the continents drift about on the surface of the Earth, for which two British scientists were awarded a Nobel Prize, may not yet have made new businesses, but has made every practising geologist wiser and thus more effective.

Certainly it is anomalous that a country with such a proud record in the academic sector of the research enterprise should so consistently fail to perform as well economically as its natural competitors. The pickle in which British research, and the academic sector thereof particularly, now finds itself has arisen because of the expectations — sometimes demands — that it should become the handmaiden of a resurgent manufacturing industry while somehow continuing to win Nobel prizes.

The misunderstanding is profound. The academic sector is mostly preoccupied in research with projects in natural science for a good reason. They, of necessity, are projects that most con-

veniently confront young people with the literally unknown. That is why, for example, a few handfuls of young people in Britain now are being educated as researchers by using telescopes and other instruments to study stars and other objects in the sky.

Eventually, each will write the equivalent of a short book on the subject; if he or she is lucky, or perhaps unusually imaginative, it may even be possible to advance an explanation of what has been seen that commands general respect; and then, because there are very few permanent research positions, each will probably have to leave astronomy for good. So why not 'train' these people, from the outset, in some field in which Britain has an economic interest — making better computer chips, for example? That is what the complainants (and the Thatcher governments especially) have been asking.

The short answer is that 'training' is the wrong word. Education in research is not about subject matter or techniques, important as they are, but about answering questions not yet asked.

There are three more polite responses. First, most people educated as professional astronomers are found to have acquired excellent computer skills and are, as it happens, eagerly sought after by the computer industry and in the application of computer technology as if they had been trained in computer science.

Second, despite the growing competition for funds and ideas within even the academic sector of the research enterprise, there is still a sporting chance that a young person will have a problem to himself for the two or three years it requires, at the beginning, to get his mind around it and to demonstrate his capacity to grapple with it. (In computer science, by contrast, problems are moving targets defined by what competitors have done — and equipment costs are usually greater.)

Finally, the designation of astronomy and other such fields as the substrate of education in research has the advantage that they are timeless: they are likely to serve as well in the education of researchers in whatever succeeds computer science as they are in supplying experts to that industry now.

Insistence, especially in the decade past, that the academic sector's research interests should be more 'relevant', or 'applicable', is not merely a misunderstanding, but an offensive misunderstanding, akin to the layman's complaint that it is beyond comprehension that some people devote their lives to the study of literature when he (or she) is used to reading novels at weekends, on

vacations or on long aircraft journeys.

An explanation for Britain's relatively poor performance in industrial innovation, rather than a scapegoat, is more likely to be found in the relatively small spending on research and development financed from their own funds by industrial companies in Britain (the chemical and pharmaceutical industries excepted). Every objective inquiry since the Second World War has come to that conclusion.

The constant drumbeat of complaint against the relevance of the academic sector's projects, echoed as it often is by the managers of industrial companies who should know better, is not merely offensive but also dangerous. The research councils in particular, but also universities, have done their best to respond, sometimes successfully, sometimes with the unproductive diversion of resources. And the argument engenders incomprehension and bad temper.

That is why Britain must urgently clarify the purposes of public support for the academic research sector, and must cultivate general understanding and acceptance of what they are. In the long run, that is the only basis for telling on what scale the academic research enterprise should operate. We stand on the principle that the training of able young men and women in research is a sufficient justification of the academic sector's existence. That the same enterprise may also contribute to the solution of more immediate industrial problems as well as to the international fund of knowledge and understanding (winning Nobel prizes in the process) must be regarded as an uncovenanted bonus.

It goes without saying that the recognition that such a principle must underlie public support for the academic sector would impose novel obligations on it as well as providing it with an extra degree of freedom. Not least, the grant-making agencies and especially the research councils, while remaining as zealous as they are now in the pursuit of excellence, would be bound also to distinguish between projects on the basis of their suitability as means of education in research.

There would be some uncomfortable consequences. The research councils, already anxious to increase the proportions of their funds spent on research grants in higher education, would find themselves compelled to transfer to universities some of the expensive managerial and service functions they now provide centrally.

Uneconomical though such a process may seem, is there not good reason why a young researcher in a team of people

successful in obtaining funds for launching, say, a new Earth satellite, should have some of the responsibility for making sure that the project is executed competently as well as for the intellectual objectives of the project? That, after all, is how innovators must conduct themselves in the real world. Why should they not begin during their education in research?

There are further implications for the recruitment of young men and women into research. Government support for research education has followed a simple pattern since the end of the Second World War. The research councils (and, in some fields such as library and information science, the Department of Education and Science directly) place in the gift of selected departments the right to appoint research students under a kind of quota system, but subject to review of the qualifications of those appointed. One consequence is that the geographical pattern is less responsive than it might be to the changing pattern of research interests.

Another, more insidious danger, is that research departments are inclined preferentially towards the appointment of their own bright undergraduates to research studentships. The better research departments in Britain are aware of the dangers of inbreeding entailed; in the past few years, the movement of graduate students from one institution to another has been deliberately encouraged. But the recruitment into research of able students from less well-known places is often haphazard, perhaps the result of personal acquaintance.

If education in research is to become the prime responsibility of the academic sector, it is unthinkable that this recruitment system can be either wise or equitable. There is a strong case for making entry into the research profession competitive (as, to some degree, it is already in France). The health of the British academic sector will not be fully restored until the award of a research studentship is once more regarded as a valuable prize. And those who succeed in the competition should have a greater degree of freedom than at present over the choice of a research department.

A precondition for such a state of grace is that the payment of research students should be much more generous than it is at present. Until the present year, the stipends paid to research students (which are tax-free, but often so small as not to be taxable) have not been very different from £3,000 a year (but there are extra payments to students living in their own houses or in expensive cities such as London). There are also extra allowances for research-related expenses.

During the past few months, several of the research councils appear to have taken fright at the difficulty of recruiting able people under these conditions. Thus the Medical Research Council announced last February that, from this October, research students on its books will receive £7,175 if they work in London and £5,590 if they work elsewhere.

The council goes some way to acknowledge that it has been partly shamed into these increases by the practice of the medical charities (notably the Wellcome Trust) of treating students more generously; it says that there will also be a reduction in the numbers of the new awards it will be able to afford. But there are many who will say that £7,175, even if tax free, is not enough to keep body and soul together in London. Newly qualified secretaries can expect to earn perhaps half as much again.

Other research councils are less generous. The Agricultural and Food Research Council, for example, offers London-based research students £6,300 a year, but only £3,700 to those living with their families. There are also curious anomalies in the system of rewards; the best way of earning a PhD is to be employed as a research assistant (when salaries are met from research grants) and to win permission to read for a research degree.

It is inevitable that those chosen as research students will compare their condition with that of fellow graduates, many of whom will be earning several times as much in their first jobs in industry or commerce, and will ask why those in whom Britain appears to have placed some trust for the future conduct of innovation continue to be treated so meanly. Continued student status may also be demeaning, and may foster the impression that education in research is not really work.

That is why the value of research studentships should be quickly increased. A doubling of present rates would be simple and equitable.

This recommendation would meet the objection that many research students would then be better paid than some in academic research who are qualified PhDs, for whom the minimum salary in a research post is now £11,399 a year (with an extra £2,000 a year in London). But that is another, if important story. The academic research profession is generally underpaid, and its work is impoverished as a consequence.

A more deliberate policy towards the recruitment of young people into research would entail changes in the pattern of training offered in higher education. In many fields, departments still cling to the old British ideal that a sufficiently bright

student can be given a problem to tackle and then, with occasional discussion and supervision, relied upon to produce a solution. Formal instruction, say in advanced techniques, is mostly anathema.

British higher education institutions will have to follow other graduate schools (notably those in the United States) in the formal provision of the background to their research studies. Otherwise, the general sense that British research graduates do not exude the expertise in their fields that characterizes, say, their US counterparts is certain to persist.

The question of how many research students there should be in a country such as Britain is not as complicated as it is often made to seem. The total number of academic and research scientists and engineers at British universities is 21,000; the polytechnics bring the total to more than 30,000.

It can only be a matter of time before polytechnics follow universities in regarding a PhD as a necessary qualification for a teaching post (how else will they become research universities?) so that even with the low wastage rate of 3 per cent a year, an annual production of 1,000 PhDs will be needed merely to renew academic staffs. (That is not much greater than the number of research students taking academic appointments in 1988 — estimated at just over 800.)

Of the total of 3,300 such people leaving British universities in 1988, more than 1,000 found jobs in British industry or government employment (roughly in the ratio of 4:1). A surprisingly small proportion (a total of about 400) are known to have taken jobs overseas. It would be prudent to plan for an increase in the numbers choosing to work overseas as the European single market becomes a reality, perhaps by a factor of three, and for a doubling of the number of PhDs employed by British industry (if the lesson that research, and the people skilled at executing research projects, eventually sinks home).

Britain should therefore legislate for an increase of roughly 50 per cent in the numbers following PhD courses in science and technology. The extra direct cost would be considerable — perhaps £100 million a year after allowing for increased stipends — and would fall chiefly on the Science and Engineering Research Council (which provides roughly 85 per cent of research studentships in science and technology). Specifically, this implies the recruitment of 5,500 new students each year and a resident population of perhaps 20,000.

Winning such an increase would not be simple. The government would argue

that such a shift in the pattern of research council spending should be financed at the expense of direct support for research. But such a response would merely be confirmation that the government concerned is not serious in its pleading that the innovation industry must be revitalized.

The issue of pay within the academic sector as a whole is a more general cause for anxiety. For much of the past 20 years, university budgets have marched in step with inflation, and academic salaries likewise. It is natural that those concerned should ask why a group of people of whom so much is expected should be rewarded so poorly. Yet at British universities, only the holders of professorships can expect to earn more than £30,000 a year, whatever their experience.

People in industry acknowledge that low academic pay makes it easier to recruit qualified people relatively cheaply. (At ICI Ltd, those joining with a PhD can expect to start at a salary of between £17,000 and £18,400, which even so is rather less than an analyst in the City of London would expect to earn at the same age.)

In the short run, low salaries for scientists and technologists may encourage industry to employ more of them, but the long-run effect may be quite the opposite. People may drift away from what they are equipped to do into management and administration, while the organizations for whom they work will be confirmed in whatever suspicions they harbour that the work of people employed so cheaply cannot be of great value.

Academic salaries in science and technology therefore need to be increased in line with what comparable professionals earn elsewhere, if necessary differently from the salaries of academics in other fields. British universities are used to paying more to clinical staffs. If science and technology are as important as is generally agreed, does not their case demand special attention?

There remains the matter of how British science should be organized, for which there is also a long history. If the financial pressure on science became apparent in the 1970s, the administrative history goes further back. Most of us forget (or have never had the opportunity to remember) how much has changed since the Second World War.

Then, there was a simple mechanism for government support of research. There were two research councils (for medicine and agriculture) and a Department of Scientific and Industrial Research (DSIR) which was the chief source of public support for research projects in the

academic sector, but which also had a direct role in applied research through its sponsorship of more than a score of industrial research associations. (Then, there was no Department of Trade and Industry, the successor of the Wilson government's Department of Technology, but a Ministry of Supply concerned chiefly with the sponsorship of defence industry and nuclear energy.)

The machinery may have been simple, but its scale was plainly inadequate for the balanced development of the British research enterprise. (The budget of the DSIR rose towards £2 million a year during the 1950s, but the Ministry of Supply, whose responsibilities for nuclear energy were later transferred to the UK Atomic Energy Authority, was already spending much more on essentially basic research.) Advice on scientific policy was provided by an independent Council on Scientific Policy, which was free to comment on any matter of scientific interest (and whose annual reports, to the Lord President of the Council, were published without censorship).

The academic sector benefited chiefly from DSIR's sponsorship of research students, but there was a steady trickle of small grants and occasional investments in much larger projects (such as the first steerable radiotelescope at Jodrell Bank). In this period, much important research in the British academic sector (the discovery of strange mesons at the University of Manchester, for example) was possible only because of equipment and financial help from private companies, by a number of private schemes for supporting research fellows (such as that run by ICI Ltd) and by marginal funds wrung from straitened recurrent grants to universities.

Two developments early in the 1960s revolutionized this system. First, there was the Robbins Report on higher education (named after the late Lord Robbins) and the Trend Report on the organization of science support (named after the then Sir Burke Trend, later Lord Trend). Robbins's chief recommendation, that the British university system should be at least doubled in scale within 15 years was enthusiastically endorsed by the then Macmillan (Conservative) government. Special attention was to be given to science and technology, notably by the singling out for special attention and support of institutions such as the Imperial College of Science and Technology.

Trend, on the other hand, provided what proved to be a handy organizational blueprint for the Wilson (Labour) government (which took office in 1964). The academic and industrial interests of the DSIR were separated, the former into the Science Research Council (now the

Science and Engineering Research Council). By then, the Natural Environment Research Council had independently been created. General policy on civil science was to be managed by a Council on Scientific Policy, conceived of (and which saw itself) as a kind of governing council for British civil science, and especially for the academic sector.

Neither Trend nor the Ministry of Technology had forgotten about industrial research. Under Mr Anthony Wedgwood Benn (now Mr Tony Benn), the Ministry of Technology developed an ambitious plan for a series of organizations intended to provide support and planning for particular industrial sectors. But by then, in the late 1960s, the government had been shaken by the sterling crises of 1965 and 1967 and had been disappointed at the outcome of its plans to revitalize the British economy by means of 'white-hot technology'. The optimism of 1964 had quite evaporated.

The attrition of British science can be traced back to that period. It was not long before the Wilson government (which had enthusiastically endorsed the Robbins proposals) grew discontented with the cost implications — especially the assumption that university teachers would have the time for research and, when appropriate, one of the research councils would provide the necessary resources.

At that point, the Labour government was attracted by the conclusions of a report of a committee under Sir Alastair (now Lord) Pilkington which had pointed out that university education was much more expensive than that provided by selected technical colleges; for that and other reasons, 26 technical colleges were designated polytechnics, providing mostly first-degree and graduate courses, but no provision was made for research. Although responsibility for the polytechnics was transferred from local education authorities to a Polytechnics and Colleges Funding Council under the Education Reform Act of 1988, and the polytechnics have now been told (in June this year) that they may choose to function as (and call themselves) universities, there is still no significant provision for research.

From the early 1970s, the annual test of British governments' duty to the academic research sector has been that of whether the science budget had increased as quickly as inflation, rather than as quickly as the number of scientists working in the universities. To make ends meet, the research councils (notably what was then the Science Research Council, whose remit is the support of the academic sector) slipped into the habit of providing expensive facilities and equipment centrally; as a result, universities

have been deprived of the challenge and the experience of managing projects of an industrial scale.

The centralization of research facilities should be reversed, and quickly, not only to release funds for research grants but also so as to make projects more responsible for their own success.

After a quarter of a century during which the British academic research sector could boast of the dramatic successes of several small research groups (in fields as different as radioastronomy, cosmic rays, plate tectonics and, above all, molecular biology), by the 1970s the research councils had begun to find that they could no longer support all the excellent research proposals put to them. This was also the beginning of a long period in which it has been exceedingly difficult for established researchers in mid-career to found research groups large enough to compete effectively on the international scene and from which new disciplines and specialisms spring.

Changing this state of affairs must be a central objective of public policy in the 1990s. Research grant applications are now judged mostly on their scientific merits and the competence of the applicants; adding the criterion of their utility, and attractiveness, to students would help to make some refusals explicable. The research system needs more freedom in broking research proposals internationally.

Lord Rothschild's plan for the reorganization of civil science, drawn up in 1971 at the request of the new Conservative prime minister Edward Heath, did little to help. One of its objectives was to devise a scheme in which government would be freed from making detailed decisions about research. A subsidiary objective was to answer the question "How much should be spent on research?"

Rothschild recommended a market solution: let there be as much research as there are customers willing to pay the cost. The recipe (adopted without delay) was simple, perhaps beguilingly so, offering what seemed to be the advantages of an objective way in which the customers (government departments standing proxy for the voters) would make objective judgements on spending at their own laboratories and, to some extent, in the research councils.

Rothschild himself was aware of the dangers. He made a great point of the need that customer departments should be equipped with strong and independently minded chief scientists, able both to strike hard bargains with contractors (usually research councils) and to appreciate the benefits of research.

Not often has this ambition been realized. The chief disappointment is that departmental chief scientists have rarely been able to persuade their departments of the benefits of research. Only when there is a government commitment to the scientific solution of a problem (such as the understanding of the causes of climatic change) does the relationship between public customer and contractor become buoyant. (Even then, research contractors may find large parts of their budgets earmarked for purposes defined externally.)

Moreover, the relationships between public research customers and contractors have mostly ranged from coolness to near-hostility. When the Ministry of Agriculture, Fisheries and Food (MAFF) decided to walk away from its contract with the Agricultural Research Council (later the Agricultural and Food Research Council) it simply walked away.

Nor was Rothschild able to make objective the criteria for telling how much British governments should spend on research in the academic sector, implicitly acknowledging that this amount is a kind of bargain between the government and the research community. Instead, Rothschild invented the Advisory Board for the Research Councils (ABRC), still extant, which gives advice to the Secretary of State for Education and Science and which differs from its predecessor (the Council on Scientific Policy) by including people independent of the scientific establishment (as well as the heads of research councils *ex officio*).

This arrangement leaves the bulk of government spending on science and technology essentially untested by informed opinion, except to the extent that the Chief Scientific Adviser in the Cabinet Office may occasionally be able to intervene. Logic suggests the abolition of the ABRC, the transfer of the academic support functions of the agricultural, medical and natural environment research councils to the Science and Engineering Research Council and the incorporation of their rumps as agencies of operational departments. Then there has to be an independent council whose chief function would be to appraise and evaluate departmental decisions in science and technology, and which would ordinarily publish its opinions except when national security would be jeopardized (which need be never). Those whose grant applications are turned down on technical grounds are right to complain that more than £3,000 million a year of government spending hardly ever receives a second opinion.

The matter is all the more important because the customer/contractor principle has become more explicit. Large

parts of the public sector research enterprise (the Atomic Energy Authority, the defence research establishments and even the National Physical Laboratory) have been given agency status, which means that they must function as if they were autonomous commercial organizations, deriving income from research contracts (mostly, but not exclusively, with government departments) and balancing their books from one year to another.

An administrative rearrangement of this kind would have several other advantages. What would be called in the United States the 'mission-orientated' functions of the medical, agricultural and natural environment councils, while remaining in spirit parts of the academic sector (and having their autonomy if anything confirmed by their status as agencies) might benefit substantially from the advocacy of departmental ministers. They would also be free to compete for funds from elsewhere, notably the European Communities.

The introduction of a new advisory council may seem a conventional reflex, but it is not. The most obvious gap in the British machinery of government in science is that of a mechanism for appraising the research and development of government departments. In principle, the Chief Scientific Adviser in the Cabinet Office has the appropriate powers. No doubt the holder of this post can exert an important influence at the margins. But it is hard to think that such a person (with a few helpers) can put a strategic stamp on the whole pattern of government spending on research and development.

Attempts in the 1980s to provide such a function by means of the committee known as ACARD (Advisory Council for Applied Research and Development) and its successor ACOST (Advisory Council on Science and Technology) have been conspicuously unsuccessful. Between them, they have produced a trickle of disparate reports on matters as varied as the export/import business in information technology equipment and secondary school science education. Questions such as the 'near-market' doctrine, emanating from the Cabinet Office in the late 1980s, which laid down that government should not support projects likely to lead to commercial products in the short term, have never been openly discussed, the ambiguities of 'short term' notwithstanding.

The new council would have something of the status in the British pattern of government enjoyed by the President's Science Advisory Council in the United States until its abolition by President Richard M. Nixon in 1971. By its freedom to tackle issues of its own choice,

and to make its conclusions public, such a council would recapture for Britain the benefits of the old Scientific Advisory Council of the 1950s.

But who would listen to it? In the long-running British controversy about the organization of science, the question of whether there should be a Minister for Science constantly recurs. The arguments on either side are strong, but not well-directed at each other. In 1981, the House of Lords Select Committee on Science and Technology argued for a part-time minister on the grounds that only such a person could command the attention of government to important matters affecting science. The Labour Party proposes that there should be a full-time minister in the Cabinet Office (who would thus be a creature of the Prime Minister).

In reality, Britain had a part-time science minister for two years in the early 1960s, when Lord Hailsham (a lawyer), who was Lord President of the Council in the Macmillan government, did the job with distinction and some flair. His success rested on his political clout — on his ability to direct his ministerial colleagues' attention to issues that his scientist advisers had persuaded him were important. But his remit did not run to defence research.

The dangers in a full-time appointment (especially of a person with a technical background) is that he or she would quickly be seen as (and would assume the role of) 'Chief Research Manager, Britain, plc'. But the present need is not for more centrally made decisions about projects, but for continuing attention to the proposed machinery for the government of science in Britain, in relation to both the changing pattern of opportunity in research and the evolution of the European Communities.

Therefore, there should be a member of the Cabinet with the responsibility for supervision of the machinery of government support for research and development. The minister would appoint the proposed advisory council, but would not be its chairman. He or she would be able to direct that the council study and give opinions on questions referred to it, and would ordinarily use it as an investigatory arm. The same minister would have responsibility (now with the Cabinet Office) for collecting statistical information.

Would such an arrangement of itself revitalize the industrial sector? Not necessarily. In the past decade, British governments have largely sought to rely on exhortation that industry should spend more on research, both in its own laboratories and in universities, but have blunted the effectiveness of their message by giving the impression that all industrial

spending is equally (and highly) meritorious. At the same time, government has set its face against direct support of industrial development (although the Department of Trade and Industry has kept in being some joint-financing schemes); the near-market doctrine applies. Tax incentives have been ruled out.

It is right that governments should not pretend to expertise in the support of particular projects. On the other hand, the near-market doctrine has been too rigorously applied (against fast-reactor development, for example, to which there are other objections), especially when other European Communities members have learned that modest government support for projects will unlock larger sums from Brussels.

Perhaps government's most effective role in relationship to industry is to disabuse it of the notion that the academic sector is failing in its duty if it does not yield a steady stream of usable invention without prompting (or development cost). Industry should learn that the academic sector is potentially a research contractor, but primarily a source of the skilled people without whom the industrial research sector cannot function — and industry itself may languish.

Government policy — it needs no new machinery — should therefore be directed to persuading British companies of their need of high skill in research and development. A few object lessons that industrial research can be profitable would help enormously.

How much would all this cost? The extra direct cost of an increased number of graduate students is the only identifiable item in the preceding suggestions. But there is a sense in which it is impossible in present circumstances to make objective assessments of what the system needs. How can the Advisory Board for the Research Councils hope accurately to judge the value of in-house biomedical research against the general need of research grants and universities?

What does stand out is that a new government will have to start where the present leaves off; why not resolve in the first year to spend only what the present planning figures allow for, letting it be understood that agencies quick to adapt to the new objectives would be the first to be allowed supplementary budgets? That might get things moving nicely.

That there is an urgent need for change to another direction cannot be disputed. It is a shame, when Britain still has so much talent in the field, that there should be so much despondency. And a waste, when so little would put things to rights. □

Japan's graduate university matures

The long-standing concern in Japan about the shortage of indigenous PhDs will be partly met by the graduate university about to graduate its first cohort of students.

Hakone

It was shown last week that all the students and most of the faculty of Japan's unique graduate school without a campus yet to call its own can still be crammed into a modest meeting hall at this mountain lakeside summer resort between Tokyo and Mount Fuji. But not for much longer. The education council of the Ministry of Education, Science and Culture (MESC) is likely on Friday this week (13 September) to have approved a plan to double student numbers to more than 250. Next spring, when the university will be graduating its first PhDs, there may also be agreement on a permanent campus at Hayama to replace the present mailing address at Yokohama.

The origins of the Graduate University for Advanced Studies lie in the heart-searching in Japan during the 1980s over the conundrum often over-simplified as: "Why is Japan outstanding at technology, but less so at basic science?" The same issue also stimulated the debate in the 1980s about 'creativity' (or the supposed lack of it). The explanation was accurately found where it might have been sought in the first place, in the pattern of research and education at Japanese university departments and in the predilections of Japanese employers.

Briefly, the great Japanese companies (whose business decisions ensure that 75 per cent of Japan's research and development is privately financed) prefer new recruits who are not free-standing scientists and engineers, but people still amenable to training. Some academics put this differently, saying that the companies prefer to recruit people who are still malleable. Whatever the best form of words, the effect is that there are many masters' degrees, but many fewer homegrown PhDs — fewer even than in Britain (see page 105). Because the best-equipped university departments are often those enjoying industrial research and development contracts, a master's course may often be seamlessly connected with the induction to a lifelong career in industry that usually follows.

The Graduate University is MESC's first response, but it is generally agreed that the university would not so quickly have come into being without the persistence and guile of Saburo Nagakura, its first president, who was the director of the Institute of Molecular Science at Okazaki until 1986. Nagakura, unlike most Japa-

nese, lets his ambitions show — he says that he wants his university to grow until there are "at least 1,000 students", for example. It is easy to imagine him not taking "No" for an answer. One colleague said this week that while he may not be universally popular, "he's necessary for Japan".

The idea of the university is simple, the practice a little more complicated. Over the years, Japan has acquired a group of basic research institutes (of which that best-known internationally is the National Institute for High-Energy Physics, or 'KEK', at Tsukuba) which exist to provide cooperative research facilities for the national universities, but whose permanent members of staff are inevitably isolated from teaching. So why not link them with a body of students recruited from the universities, but including a sprinkling from overseas? The notion has been in the air since 1982, but became a reality only in October 1988.

There are now seven parent institutes. As well as Nagakura's old institute at Okazaki, the two other national institutes on that site (for basic biology and physiological studies) have joined up. So too have the National Institute of Genetics at Mishima, the Institute for Statistical Mathematics at Tokyo, the National Museum for Ethnology at Osaka and the high-energy physics laboratory (which, among other things, operates Japan's hugely successful electron accelerator and is building the world's most energetic synchrotron radiation source). The hope is that further research institutes will accrete as the university grows.

The fields to which students are assigned are not co-extensive with those of the member-institutes, but are, rather, subsets of them selected for their usefulness for education in research. Thus students assigned to KEK do not join teams making observations in high-energy physics, but are involved with the design of accelerators based on novel techniques or on the application of accelerator technology outside high-energy physics.

The student body is thus scattered for most of the time over more than 200 km of Japan, but the sense of being detached from the institution is overcome by meetings of the whole group at intervals of six months. One such event had brought them to this beauty spot last week, together with a sizable number of the 200 members of the faculty and various speakers on sci-

ence and its social context. Nagakura explained the eclectic nature of the programme by reminding people that Carthage had been a civilization whose prosperity had been much envied at the time, but whose culture had done nothing for Carthage or its successors. Japan, he declared, did not want that reputation.

Can this unique university help to secure that goal? There are some obvious difficulties, not the least of which is that, if students in general are diverted from PhD courses by the eagerness of companies to employ them, and if the regular universities have first claim on the loyalties of the others, may not the graduate university be disadvantaged in the competition for bright young people?

That seems to be a worry, but not yet a serious one. Nagakura hopes that the attractiveness of the facilities on offer, as well as the reputations of faculty members, will carry the day. One faculty member acknowledges, however, that it may have been necessary to accept lesser qualifications (the equivalent of a two-year master's course) than would the universities of Tokyo or Kyoto. But, he says, members of the faculty also use their personal influence with their academic colleagues at the national universities to recruit able students (whose stipends of ¥140,000 a month — roughly \$1,000 — are to be increased next year to ¥160,000). For what it is worth, casual conversation with those concerned proves that here, as everywhere, they are as bright as paint.

Even so, the first graduations next March will be a sign of the way the wind is blowing. A dozen students will have written and defended dissertations, and will have begun to get jobs for themselves. Most, it is believed, will gravitate towards academic life, but that provokes another question on everybody's mind: if Japan has been short of homegrown PhDs, it also still lacks a sufficient supply of postdoctoral positions in which people can build reputations for themselves (and to which those who have gone overseas for a spell can return while hatching more permanent careers). Will that be Nagakura's next cause? But now, he is too busy planning the new campus at Hayama. Although nothing will be ready for occupation until 1994, the university already knows what it will then be getting. Japan may have been slow to appreciate the importance of graduate students, but it is as deliberate as always. **John Maddox**

The silence of the past

Robert A. Foley

HOMINIDS have been in existence for up to seven million years, bipedal for at least four million years, and have had much larger brains than those of apes for about two million years. During those two million years they made tools, colonized much of the world and lived in large social groups¹. But according to work by Noble and Davidson, published in *Man*², it was only in the last 40,000 years, some 60,000 years after the appearance of anatomically modern *Homo sapiens*, that language evolved.

What is surprising about the proposal is that it runs counter to studies of animals that have unearthed increasing evidence that social mammals and birds have complex communication systems³, that they use them to discriminate between individuals and assess each other's status and condition, and that they employ calls as signs with external referents. Animals using acoustic means of communication are highly sensitive both to other individuals' behaviour and to the context in which that behaviour occurs.

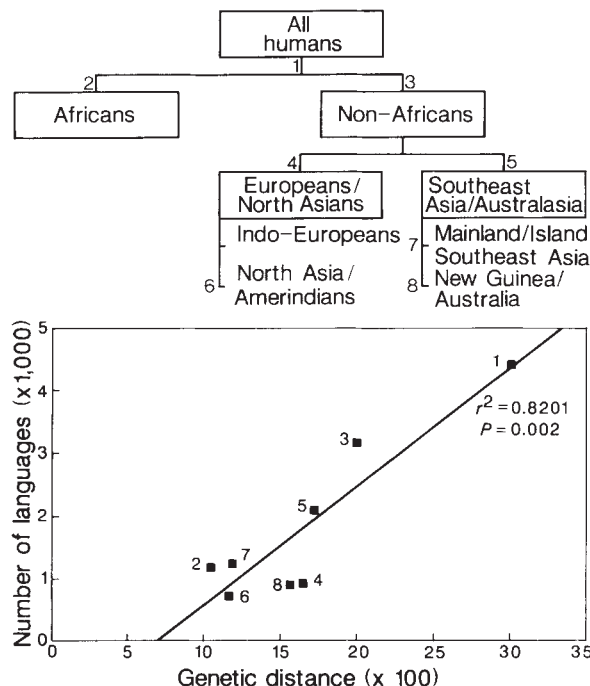
The greater understanding of the sophistication of animal communication has come about partly from the recognition that communication systems are strongly embedded in social behaviour and social organization³. Primates in particular are highly social, and so it is to be expected that they should be among the most developed in terms of communication systems. If this is true, it is all the more improbable that the early hominids did not have some form of language.

In the face of the comparative evidence, then, the claim that modern human language is of recent origin is unexpected at the very least. The argument has two lines of support — the inter-relationship between language and symbolism, and the evidence for symbolism in the archaeological record.

Noble and Davidson propose that the principal characteristic of language is its symbolic nature, consisting of arbitrary symbols that designate other objects or actions. Although animals have been shown to have considerable signalling ability, Noble and Davidson consider that animal communication lacks the symbolic potential that underlies human language. Thus they argue that human language is unique, and that it is closely bound up with the notion of culture, itself a concept defined primarily in terms of its symbol-bearing capacity. If they are right, then the gulf between human speech and animal communication remains wide despite the extent to

which other animals use signs and signals in flexible ways. The authors claim instead that the existence of language can be inferred only if there is evidence for true symbolization in the archaeological record.

They adopt a sceptical and minimalist



There is a strong relationship between human genetic variation and linguistic groups¹¹, showing that genes and languages may well diverge in similar ways. If genetic diversity found in each of the successive major groupings (top) of living human populations is plotted against the number of languages spoken by those groups, a strong linear relationship is obtained (bottom). The numbers refer to the nodes of genetic relationships. Outliers such as Pacific islanders and Africans may reflect geographical effects, such as island isolation or habitat diversity, as well as historical factors. (Data from refs 9 and 11.)

approach to this question. As it is only in the Upper Palaeolithic, about 40,000 years ago, that forms of repeated iconic structures (engravings, sculptures and ultimately cave art) appear, they conclude that this is the only true evidence for language. Before that, all artefactual material can only be described as having forms closely related to their functions, and therefore lacking the key criterion of symbolic content.

Although based on negative rather than positive evidence, this is a challenging reinterpretation of the evolution of human behaviour. In place of the gradual accumulation of human traits over millions of years, with strong continuities across the non-human primates, the model is one in which early hominids were essentially bipedal apes lacking the central cognitive characteristics of mod-

ern humans. Only in the final stages of human evolution did language provide the revolutionary catalyst that resulted in the enormous radiation and diversification of human cultures and populations that has occurred in the past 30,000 years. Furthermore, the late advent of language would mean that a major behavioural change was decoupled from the anatomical changes associated with the origins of modern humans.

Is there any other support for this view? Certainly the idea of a late origin of speech and, by implication, human language, is not new; it has long been argued that Neanderthals were incapable of modern speech⁴ (although this has been disputed⁵). Other lines of evidence are suggestive. Analyses of hominid palaeontology emphasize the fact that anatomically modern humans represent a considerable departure from other more archaic hominids, some of which, the Neanderthals for example, were contemporary or later. In association with genetic evidence, this point has been used to argue that modern humans had a single (African) origin and subsequently replaced the archaic forms elsewhere in the world⁶. Again, this idea is controversial⁷, but the notion that modern humans with their characteristic gracile skulls and skeletons were radically different from other species of hominids may be in accord with the behavioural evidence. It is striking that, before the arrival of modern humans, technology was remarkably

stable over hundreds of thousands if not millions of years, and across whole continents, whereas after the onset of the Upper Palaeolithic tool forms become more variable and transitory⁸. Pre-modern human language may have been equally stable and discontinuous with modern languages, which, like modern technology, changes rapidly.

A further clue can be gleaned from attempts to reconstruct linguistic history. This subject is even more fraught with disagreements than are studies of human evolution (indeed, many linguists would say that such an enterprise is impossible), but a number of workers now think that it is possible to construct an evolutionary tree of the main language families, stretching in theory back to the original stem language⁹. Nostratic, for example, a linguistic superfamily consist-

ting of most of the Eurasiatic languages, is thought to have been spoken across a large area of Eurasia at least 15,000 years ago. Renfrew¹⁰ has suggested that this may be associated with the apparent cultural unity, known as the Gravettian, found from the Dordogne to the Urals in the archaeological record for the period between 26,000 and 20,000 years ago. Perhaps more startling is the link proposed between linguistic phylogeny and genetic distance (see figure). Cavalli-Sforza¹¹ has proposed that the congruence between these trees is striking, and that dates of between 60,000 and 100,000 years ago would be appropriate for the stems of each. A number of lines of evidence seem to suggest that all extant human languages have their origins in the last 100,000 years, a period associated with the origins and dispersal of modern *Homo sapiens*. Whether the origin of this stem language is the same as the origin of all human language is a more difficult question to answer.

The main problem to resolve is the disparity between the ethological, palaeontological and archaeological data. One possible solution is to disentangle language from thought. Although the external signs (calls, gestures and so on) of non-human primates may have limited symbolic content, it does not follow that the individuals involved lack the ability to think in abstract ways. The work of Cheney and Seyfarth³ shows that monkeys think more than they communicate about those thoughts, and selection for complex forms of thought is unlikely to be the same as selection for the need to communicate ideas and thoughts. To some extent this point explains the apparent 'unrevealed capacities' of apes, in that with human stimulation and in experiments they are capable of more than they are in the wild.

What may have happened in human evolution is an extension of these capacities. For much of their evolution hominoids and hominids were clearly under selective pressures for larger brains; and selection for larger brains, it can be assumed, indicates selection for greater information processing. Most of that

processing need may have been internal to the individual, with only limited requirements for communication. This may have been the case even after the origin of anatomically modern humans, and it was only 40,000 years ago that the situation changed. Selection for communication became a major factor, perhaps as a result of changed social conditions such as larger groups or more extensive networks and alliances. If this was so, then language in its modern form does represent a radical change in human evolution, and modern human languages did diversify from a relatively recent point. But it does not imply that early hominids were not capable of flexibility, cognitive sophistication or abstract and symbolic systems of thought, ASTRONOMY

or indeed that they did not have some form of language. It just means they kept their thoughts to themselves.

Alternatively, the 'languages' of the ancient hominids may have lacked something like the chomskyan restricted syntactical structure that underpins modern languages, and it is from a single linguistic stem that such structure has diversified. If Noble and Davidson are correct, the key question that remains is why at that time the strong silent hominids who had done so well for so long should have been supplanted by our loquacious ancestors. □

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Laser beams untwinkle stars

Fritz Merkle

LASERS are coming to the aid of astronomers by creating artificial 'guide stars' that are used as reference points in adjusting the adaptive optics of specially designed instruments. In two experiments described by Primmerman *et al.* and Fugate *et al.* on pages 141¹ and 144² of this issue, powerful pulsed lasers send their light high into the Earth's atmosphere from where a tiny portion is scattered back to be detected by a telescope (Fig. 1). In fact, the experiments are several years old but were performed under the cloak of military secrecy, lifted only 3 months ago in time for the results to be discussed at the annual meeting of the American Astronomical Society. But

why, when the sky is already covered with billions of real stars, should astronomers become so excited by the creation of additional artificial ones?

Thirty-eight years ago, Horace W. Babcock suggested³ the construction of an adaptive optical correction system to overcome the degradation in astronomical image quality caused by the effects of atmospheric turbulence. The effects, attributable to the mixing of warm and cold air layers, are one of the main limitations for astronomy from the ground. The resulting twinkling of the stars can be considered as one of the biggest of the obstacles that astronomers have to face. ►



FIG. 1 Beam me up: artificial guide star created by a pulsed copper-vapour laser high over New Mexico. (Picture courtesy R. Q. Fugate.)

1. Klein, R. G. *The Human Career* (Chicago University Press, 1989).
2. Noble, W. & Davidson, I. *Man* **26**, 223–254 (1991).
3. Cheney, D. L. & Seyfarth, R. M. *How Monkeys See the World* (Chicago University Press, 1990).
4. Lieberman, P. *On the Origins of Language* (Macmillan, New York, 1975).
5. Falk, D. *Am. J. phys. Anthropol.* **3**, 123–132 (1975).
6. Mellars, P. & Stringer, C. (eds) *The Human Revolution* (Edinburgh University Press, 1989).
7. Wolpoff, M. H. in *The Emergence of Modern Humans* (ed. Trinkaus, E.) 97–141 (Cambridge University Press, 1989).
8. Foley, R. *Antiquity* **61**, 390–392 (1987).
9. Ruhlen, M. *A Guide to the World's Languages* (Stanford University Press, 1987).
10. Renfrew, A. C. *Cambridge archaeol. J.* **1**, 3–23 (1991).
11. Cavalli-Sforza, L. L., Piazza, A., Menozzi, P. & Mountain, J. *Proc. natn. Acad. Sci. U.S.A.* **85**, 6002–6006 (1988).

Astronomical images are intrinsically blurred because of diffraction at the edges of the telescopes' optics. Although increasing the aperture size reduces this limitation, the images so produced in practice are merely brighter. Their resolution is no better than that from a telescope 10–20 cm in diameter (0.5–1.0 arcseconds, equivalent to a resolution of 2.5–5 mm at 1 km) owing to the atmospheric spoiling. This waste of potential resolving power will become greater with the next generation of telescopes with apertures as large as 10 m.

Adaptive optics, however, allows one to correct all aberrations the lightwave encounters on its way from space to the astronomical detector. A wavefront sensor measures the optical distortions which can then be nullified by deforming a flexible mirror in the telescope. To be effective, the corrections have to be made up to several hundred times per second, depending on the observing wavelength and atmospheric conditions. The result will then be diffraction-limited images as if there were no atmosphere above the telescope.

Babcock's proposal seemed to be forgotten until the early 1970s, at which time the propagation of laser radiation through the atmosphere became of military interest, notably in the Strategic Defense Initiative (SDI or 'starwars'). But while that research continued in secret, astronomers considered the solu-

tion to their difficulties lay in going into space. The Hubble Telescope is the crowning achievement of that approach. Only in the past 8–10 years has progress been made in the non-military use of adaptive optics. The first successful tests were performed in 1989, using telescopes operating in the infrared wavelength range^{4,5} (Fig. 2). But although the technology already existed in the SDI laboratories, most of it had to be reinvented, owing to classification.

In any case, adaptive optics is not so easy to apply to astronomical imaging. To make any correction, one must first measure the error. Most interesting astronomical objects are too faint for correction with adaptive optics. In some cases, there are bright stellar objects nearby in the field of view. These can be used in measurement of the disturbed wavefront if their light travels through the same atmospheric layers. In the infrared range, at wavelengths greater than 5 μm , the whole sky is covered by sufficiently bright objects, because the reference need not be so close to the target object. But for shorter wavelengths, and, in particular, in the visible range, only a small fraction of the sky is suitably covered.

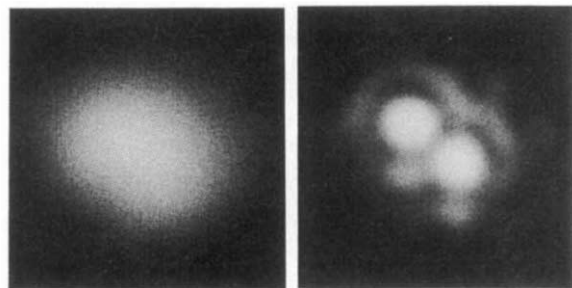


FIG. 2 A distinct improvement: by adjusting their telescope's optics, ESO astronomers nullified atmospheric distortion to obtain a clear image of the binary star HR6658.

In 1985, the French astronomers Foy and Labeyrie proposed⁶ the futuristic technique of artificial stars to overcome this shortage of reference stars. Tests in 1987 seemed to indicate its feasibility⁷. The artificial star is generated by focusing a powerful laser beam in a distinct layer of the atmosphere. Rayleigh scattering in the lower atmosphere or resonance scattering in the mesospheric sodium layer at 80–100 km then creates a bright point of light which is used in wavefront measurement. The astronomical imaging and wavefront sensing processes have to be rapidly alternated to avoid polluting the image detector with light from the artificial star.

What was considered a dream by many astronomers now turns out to be reality. Indeed, the first classified tests in 1983 demonstrated the feasibility of



Only one earthquake

GHOST forests of western red cedar along the coast of Washington state bear witness to a cataclysmic earthquake that struck about 300 years ago. They also bear a reminder for the 10 million inhabitants in the region of the future devastation that may threaten there. Tectonic convergence at the Cascadia subduction zone along the coast of northern California, Oregon, Washington and Vancouver Island is proceeding at a rate of 2.5–4.5 cm yr^{-1} , comparable to the convergence at the seismically active Nankai trough off southwest Japan, yet the zone is completely quiescent. Evidence such as the ghost forests suggests that the Pacific and North American plates are rigidly locked and accumulate enormous amounts of strain that is released episodically in massive earthquakes. The last such is dated by B. F. Atwater and colleagues on page 158 of this issue to the years 1680–1720. This date, 60 years or more following the European colonization of Massachusetts, comes as no surprise to those following developments in the study of prehistoric earthquakes along the west coast of North America. The real achievement of Atwater *et al.* is to show, through impressively precise radiocarbon dating of the timber, that two forests separated by 55 km perished at the same time, owing to sudden subsidence of coastal lowlands and immersion of the trees in salt water. From this it seems that a single powerful earthquake was responsible, not several smaller ones. If the authors can extend their results to ghost forests 100 km or more apart, it would show that the last event was a 'great' earthquake of magnitude 8–9 — almost as powerful as the one that rocked Chile in 1960. The comparison is not idle: the Chilean earthquake stirred up 10-m tsunami waves that left coastal sand deposits similar to ones found along the Pacific northwest.

Roland Pease

Rayleigh guide stars², and one year later guide stars were made in the mesospheric sodium layer. Then in 1988, Primmerman *et al.*¹ succeeded with real atmospheric compensation using adaptive optics with a Rayleigh guide star on a 0.6-m telescope adjusted at a rate of 2.5 Hz. And in 1989, Fugate *et al.*² achieved this even for a 1.5-m telescope at a bandwidth of 30–63 Hz. The teams applied their systems also to obtain corrected images in the visible wavelength range of objects like the star α CMi (Procyon) and the binary 53 ξ UMa.

It is encouraging to realize that adaptive optics and the lasers needed to generate guide stars can be built into nearly all existing telescopes. Even older telescopes may become state-of-the-art observing tools once more. And clearly, astronomers should ensure that it can be integrated into the next generation of telescopes. Large telescopes require multiple guide stars because the light from these relatively nearby beacons travels along divergent paths, unlike the parallel paths of real star light. Such

multiple guide stars will further complicate the system and necessitate greater laser power, which seems to be the main limitation at this time.

The increased complexity in wavefront sensing may be overcome by the progress recently reported by Angel *et al.*⁸ and Sandler *et al.*⁹ applying neural networks (self-adjusting electronic circuits) to adaptive optics. Not only may neural networks simplify the wavefront sensing and correction algorithms, but also their learning capabilities may allow the system to match itself in an optimum way to the atmospheric conditions. □

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Language of the genome

P. N. Goodfellow and L. Sefton

If language is a sensitive indicator of social change then genetics is undergoing a revolution. Where once human geneticists did experiments, they now implement programs and instead of using somatic cell hybrids, DNA probes and other reagents, they now create and exploit resources. These changes have been wrought by the 'genome project' and the program-speak is a reflection of the new approach. Another indication of change is the threatened demise, at least in its present form, of the Human Gene Mapping (HGM) workshops. For the past 18 years, from the first workshop to the tenth, the meetings have reflected progress in mapping the human genome and provided a forum for international collaboration as well as dispute. This tradition was continued at the 11th workshop, held last month in London*.

The main function of the HGM workshops is to compile all the available data on the human genome and to arrive at a working map that can be used as the basis for future research. This task has become so large that a computer data base is mandatory and the Genome Data Base (developed by the Welch Memorial Library at Johns Hopkins University) is fulfilling this role admirably. A secondary function of the workshops is to agree on the language that is used to describe the genome, for example to

ensure that all genes and DNA segments have unique names.

As at previous meetings, descriptions of the number of genes and DNA segments placed on the human map increased exponentially; the grand totals for mapped genes and DNA segments now stand at 2,316 and 6,831 respectively. This increase is to be welcomed, not least because the estimated number of human genes is also edging upwards. Analysis of chromosome III in *Saccharomyces cerevisiae*¹ and the sequencing of several cosmids from *Caenorhabditis elegans* (J. Sulston, MRC, Cambridge) is revealing more genes than expected. Extrapolation from these organisms could imply the existence of 100,000 genes in man. Many of them may be clustered in gene-rich areas of the genome. One notable example is the much-studied major histocompatibility (MHC) region: in four megabases of DNA there are over 74 genes, some of which are so closely spaced that they overlap (D. Campbell, MRC, Oxford). Even in regions of the genome previously thought to harbour a single large gene, genes are being found within genes like Russian dolls; specific examples include a potential gene in *F8* (ref. 2), three genes in the first intron of the *NF1* gene³ and the discovery that *TIMP* lies within an intron of the synap- sin gene⁴.

In most cases it is not known if these intimate genetic relationships have a

biological meaning. But the importance of gene order in the β -globin complex, and the functional relationship between genes in the MHC region, shows that gene mapping is likely to continue to provide clues to function. These clues may be reinforced by comparing the gene maps of different species — if the physical relationship between genes is not conserved in different species, chance is the probable cause of their juxtaposition; necessity is suggested by conservation of gene order. A spectacular example of conservation is the homeobox gene cluster in which gene order is conserved between insects and mammals⁵. With the proliferation of 'genome projects', and the ease with which DNA markers can be ported across species, comparative gene mapping will play an increasing role in genetic analysis.

The primary social justification of the 'genome project' is the expected contribution to mapping and cloning of genes of medical importance. Already it is clear that this benefit will accrue rapidly; in the past year the genes responsible for Marfan's syndrome⁶, familial adenomatous polyposis coli^{7,8} and several others have been cloned. At the meeting, the probable cloning of the X-linked Kallmann gene was described, in detail, for the first time⁹. The symptoms of Kallmann syndrome include a bizarre conjunction of anosmia (inability to smell — this can be tested by asking patients to eat soap) and small gonads caused by failure to produce sufficient gonadotropin-releasing hormone (GnRH). Anatomical investigation has suggested that the underlying defect is a failure of the migration of the olfactory and GnRH-synthesizing neurons from the olfactory placode in the brain. Consistent with this hypothesis, the gene cloned by R. Legouis and colleagues encodes a protein with distant similarity to known cell adhesion molecules (ref. 9; A. Ballabio and colleagues, personal communication).

The HGM workshops have always been a sensitive indicator of the future directions of human genetic research. At HGM 11 the first signs of results from the global approaches to genome mapping were evident. Large contigs, collections of cloned DNA fragments in the same order as they are found in the genome, have been constructed for the terminal part of the long arm of the X chromosome in YACs (yeast artificial chromosomes)¹⁰, and several groups have used the fingerprinting techniques¹¹, developed by the Cambridge nematode group, to make cosmid contigs^{12–14}. Similarly, partial sequencing of complementary DNA clones, in an attempt to identify all expressed genes, is a growth industry (ref. 15; R. Sibson,

*11th Human Gene Mapping Workshop, London, 16–23 August 1991.

Gold fever

Will the next gold rush take hopeful prospectors to the frozen wastes of Antarctica? K. A. Meeker *et al.* report (*Geophys. Res. Lett.* **18**, 1405–1408; 1991) that the volcano at Mount Erebus, on the Antarctic island of Ross, spews out tiny grains of gold. Particles as large as 20 μm have been found at an altitude of 8 km above the summit, and larger grains have been found in ice a dozen or so kilometres away. Gold emission from volcanoes is fairly well known, but never before has the metal been found in particulate form. Holders of gold bullion need not fear, however — Mount Erebus is putting out only an estimated 60 grams a day, and in windy weather, the authors posit, grains could be dispersed as much as 1,000 km away from the volcano.

Thereby hangs a tail

MYOSIN is a protein that has given employment to many; it has an active portion (the heads) for biochemists and an inert (structural) element (the rod backbone) for biophysicists. The isolated rod is insoluble in low salt, forming paracrystals with the structural character of the thick filament core of muscle. S. J. Atkinson and M. Stewart (*J. Cell Sci.* **99**, 823–836, 1991) have expressed pieces of the rod in *E. coli* and find that they form the α -helical coiled-coils seen in the native protein. Deletion of the 'skip residues', which interrupt the heptad motif characterizing the coiled-coil, makes no difference to speak of in structure or solubility, but annihilating the C-terminal end generally prevents the formation of insoluble paracrystals. This is also the experience of H. R. Kalbitzer *et al.* (*Biochemistry* **30**, 8083–8091; 1991), who used NMR to show that the C-terminal residues (nine in one chain of the coiled-coil and twelve in the other) form a freely mobile tail, not part of the rigid rod. They suggest that its function is to pack like cement into the crevices of the filament.

Sesame sauce

VEGETARIAN food can be dangerous, as evidenced by a case of a young woman who found herself in hospital after tucking into falafel (deep-fried balls of chickpea paste). M. K. Kagi and B. Wuthrich traced the cause of violent nausea, diarrhoea, inflammation and rashes to an allergy provoked not by the falafel itself, but by the sesame seed paste used to make an accompanying white sauce (*The Lancet* **338**, 582; 1991). Screening showed that the 23-year-old patient was allergic to sesame seeds in a variety of foods, although potential allergens are usually inactivated by heating — so vegetable oil and baked burger buns are quite safe for sesame sufferers.

MRC, London; C. Auffrey, Paris). Unfortunately, many of the old problems are still with us, despite the introduction of several promising techniques^{16,17} — it is still difficult to find genes in cloned DNA (the Huntington gene, for example) and computers are still learning how to recognize genes in a DNA sequence.

The construction of genetic maps can seem tedious, especially to those who use the fruits of genetics but are not themselves geneticists. Yet without maps there is no genetics. The construction of the human gene map is an essential undertaking for biology and medicine. It is so important that all should have access not only to the maps and their

products such as genes and sequences, but also to the process of construction of the maps. As described in the news pages of *Nature*¹⁸, there are plans to change the HGM workshops to make them easier to organize and to cope with the exponential increase in both quantity and quality of data. We wish to add our voices to those who argue that whatever format is devised, all should continue to have equal access to the map and its construction. The language of the genome must be universal. □

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VISION

Polarizing the world of fish

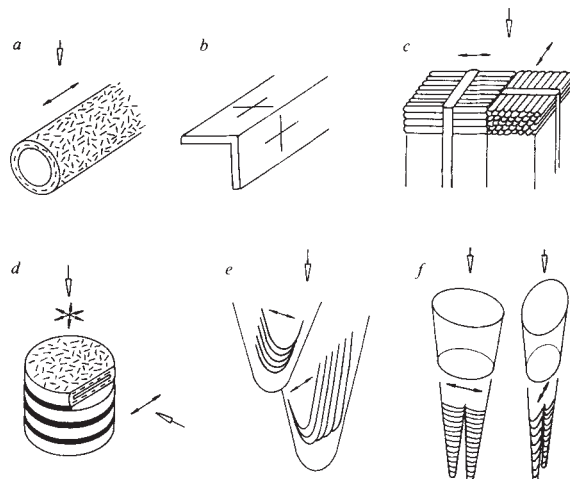
Michael F. Land

WE are especially intrigued by the senses that we lack. Echolocating bats, electro-sensitive fish and insects with ultraviolet vision all make us a little envious of ways of apprehending the world that are denied to us. The ability to detect the direction of polarization of light is another such opaque sense, and although it has been known for nearly fifty years that bees use it as a navigational aid, the possibility that our vertebrate relatives might also have this capacity is a more recent idea. The

evidence that both birds and fish can discern the pattern of polarization in the surroundings is mounting, and on page 161 of this issue¹ Cameron and Pugh describe a particularly convincing behavioural demonstration of this capability in the sunfish.

The authors suggest a plausible mechanism for polarization detection, based on the shape and orientation of the double cones of the fish retina. This is important because vertebrate rods and cones are not, in general, organized in a

Ways of achieving polarization selectivity. *a*, Microvillus is intrinsically selective as more molecules are aligned along the structure than across it. This is more obvious in a square section *b*, where twice as many must be aligned parallel to the long axis compared with either orthogonal direction. *c*, Arrangement of microvilli in a pair of octopus receptors. *d*, Disks in vertebrate receptors are not selective to polarized light from the normal (end-on) direction, but are selective from the side. *e*, In anchovies the lamellae in the cones are stacked vertically with adjacent short and long cones having orthogonal orientations. *f*, Suggestion of Cameron and Pugh¹ for the sunfish: the cone inner segments act as elliptical wave-guide polarizers, conveying selectivity on the double cones beneath. Open arrows indicate light direction and thin arrows the plane of polarization selectivity.



way that would allow this, hence our own insensitivity. To detect a photon of light polarized, say, horizontally, a rhodopsin molecule must have the crucial double bond of the retinal group aligned in the same direction. Obviously, if all the molecules in a particular receptor were aligned in that direction, then the cell would respond to horizontally polarized, but not vertically polarized, light. Another cell might have its molecules arranged orthogonally, and the pair could generate between them a signal resolving polarization direction. In many invertebrates this is what happens (see figure). Thirty years ago it was shown that the finger-like configuration of the microvilli in the photoreceptors of the octopus eye automatically ensures that most of the receptor molecules will be aligned in the direction of the microvilli². Furthermore, the receptors in the octopus and squid are arranged so that alternate receptors have their microvilli at right angles to each other, so the whole retina acts as a polarization analyser. Vertebrate rods and cones do not have aligned microvilli, but instead have disks in which the photopigment molecules are free to rotate in the plane of the disk membrane. For this reason no particular plane of polarization is preferred to any other, and thus there is no basis for polarization sensitivity.

There are two possible ways this insensitivity might be overcome. One is to lie some of the receptors on their sides. The photopigment molecules are then aligned in the plane of each disk membrane, which is at right angles to the receptor's long axis. Something like this is found in anchovies, in which the cones have their disk membranes lying almost parallel to the cells' long axes, not across them. Adjacent cones have orthogonal membrane stacks, forming a basis for polarization sensitivity³. The other possibility is to provide each receptor with some kind of polarization filter in front of the photopigment, and this is the mechanism proposed by Cameron and Pugh¹. The retinæ of fish have fused pairs of cones in which two outer segments (containing the photopigment) arise from a single inner segment whose cross-section is roughly elliptical. The authors argue that elliptical light-guides can have polarizing properties and that the inner segments can therefore behave as filters for the outer segments. The circumstantial evidence is powerful. The double cones are arranged in an orthogonal array — reminiscent of the octopus receptors — and the planes of maximum behavioural polarization sensitivity do correspond to the axes of that array; furthermore, the spectral sensitivity of the effect is the same as that of the double cones, and no other receptors.

The weak link in the argument at this

stage is the mechanism itself. What physical principle of waveguide optics needs to be invoked to produce an adequate degree of polarization from a structure with the dimensions of a cone inner segment? The experiments used by Cameron and Pugh as a basis for a model (from ref. 18 in ref. 1) refer to birefringence, and although this may result in polarization, it does not necessarily result in filtering. Polarization filtering might result from differential mode-shedding in fairly wide fibres, but the size of the effect is uncertain. The important point is that some physics needs to be done to determine whether the rather short cone inner segments can do the job required of them.

Finally, what use is polarization vision? On land its two known functions are as a compass, the pattern of sky polarization acting as an alternative to the Sun's position, and as a means of detecting reflecting surfaces — especially

SEMICONDUCTOR PHYSICS

Sure plays a mean pinball

S. Washburn

ELECTRONIC games have long since displaced the old mechanical entertainments in amusement arcades. But a reverse process now seems to be occurring, with the description in *Physical Review Letters*¹ of a microchip designed along the lines of a pinball machine. Peppered with impenetrable submicrometre indentations, the chip is used to study the wayward motion of the deflected electrons.

With the advent of very-small-scale

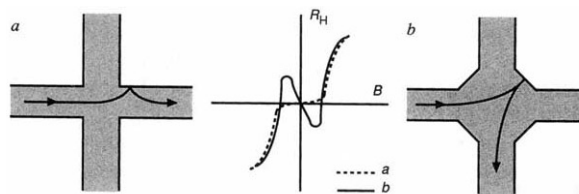


FIG. 1 Sketches of Hall resistance (R_H) measurements in small samples (centre) and the billiard ball trajectories (a, b) that cause the unusual response. Current flows from left to right and the Hall voltage is measured between the vertical ports. The magnetic field points out of the page so that the Lorentz force is towards the top of the page.

lithography for crafting electronic circuits with features less than a wavelength of light across, a great deal of research and speculation has gone into the practical uses of some quantum mechanical interference effects that can arise. The quantum interference is quite fragile, however, and the speculation was almost entirely needless as the energy needed to destroy it is so minute (10^{-5} V). Recently much more robust features have appeared that are classical in origin. They are analogous to playing pinball or

water — as these polarize light⁴. Under water there are several possibilities, but a particularly attractive idea comes from the fact that the silvery sides of fish, whose most important function is camouflage⁵, do themselves polarize light to some extent. It was suggested as long ago as 1965 that an animal with a polarization analyser could use this effect⁶, either for predatory purposes, or perhaps to allow one fish to keep track of another in a shoal. □

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billiards (depending on the shape of the transistor) with eccentric spheres. Because the classical laws of motion do not wither at higher temperatures, the only limits on the use of the new resistance features appear when the basic transport mechanisms change. The analysis and explanation of the resistance measurements rely on simple rules, but owing to the transistors' complicated shapes, the results can be rather exotic.

An electron will fly directly between

two differing electrical potentials as a result of the force on the charge caused by the electric field E . If there is a magnetic field B perpendicular to the electric field between the potential points, however, there is a force at right angles to both the electric and magnetic fields, and the electron's motion becomes a closed orbit with a radius $r_c = mv_F/eB$ (where v_F is the electron's velocity, m its mass and e its charge) that drifts along the direction of the electric field. If it reaches one of the potential probes, it can be recorded and thereby contribute to the measurement of the resistance R of the medium in which it moves.

The orbit and all of the motion result from the classical Lorentz force $F = e(E + vB)$. The cataloguing of such trajectories has been performed in extremely pure single crystals of metal². More recently the same kinds of effects

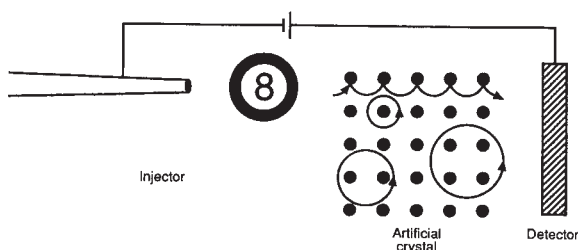


FIG. 2 Electronic pool: with isolated potential barriers, electron trajectories might skip through the obstructions to reach the detector. But certain magnetic fields will give some closed orbits, pinned to one or a few of the barriers.

have been showing up in small-scale transistors. The classical orbit of the carriers can be mapped by resistance measurements. One example is that of electron focusing³, in which electrons injected into a transistor through one small terminal are turned back towards a neighbouring terminal by the Lorentz force. Measuring the current at the exit, one finds peaks when the orbits match the focusing condition $r_c = L/2j$ (where j is some integer, L the terminal separation). With $j=1$, the electron's path between the terminals is a single arc. When j is greater than one, the electron bounces against the side of the device, executing $j-1$ skips before reaching the exit port — the trajectory resembles a billiard ball hopping along the cushion of a sloping snooker table.

When R is measured by probes on either side of a current-carrying channel, it is mainly the Lorentz force that is measured. In large samples, the carriers scatter frequently from randomly situated impurities as they trek across the sample, and this 'Hall' resistance R_H is proportional to the Lorentz force, $R_H = B/ne$ (n is the density of carriers). This is true because the random scattering makes all trajectories tend in the direction of force. In Hall measurements on samples much smaller than the mean-free-path length between scattering events, however, R_H behaves very differently. In fact one can obtain a wide variety of dependences $R_H(B)$ by changing the shape of the Hall probes. Reducing the scale of the probes and main channel to a few hundred nanometres reduces the Hall resistance to $R_H = 0$ for small magnetic fields, because the Lorentz force is not strong enough to bend the electron's trajectory into the voltage probe before it escapes down the channel (Fig. 1a). Placing a mirror at the corner of the voltage and current probe (Fig. 1b), one can force most of the carriers to reflect into the opposite probe so that R_H even has the wrong sign⁴. The dominance of such reflections has led to an instructive analogy between the electrical transport and the game of billiards⁵.

Now Weiss *et al.*¹ have managed to make convincing measurements on sam-

ples indented with regularly spaced holes. The array of holes mimics the potential imposed by crystalline structure (Fig. 2), or in their more fanciful classical metaphor, a pinball machine. The electrons scatter from the potential islands just as they would from impurities (which are present but have much weaker potentials), so that a model of the transport looks rather

like a map of a pinball game. The principal effects of the artificial potential are, first, to reduce the overall mean free path length of the electrons and, second, to provide pinning sites where the cyclotron orbits can get hung up. The first effect adds a constant amount to $R(B)$ and the second leads to peaks of resistance corresponding to the capture of a significant fraction of the electrons in the pinned orbits. At much lower temperature, the quantum-mechanical phase coherence of the electrons spans distances greater than the separation of the dots and one might expect that the resistance could reflect quantum-mechanical behaviour in the imposed 'crystal' potential.

One should not be fooled by the apparent simplicity of the classical rules governing the electron transport: an arbitrarily small change in the incoming trajectory can make a huge difference in the point at which the electron emerges. This critical dependence on the initial trajectory makes the response essentially unpredictable or chaotic in many situations^{6,7}. The use of very-small-scale lithography and very high-quality materials allows the classical motion of electrons in a magnetic field to dominate the resistance even at rather high-energy scales (10^{-2} V), which are well within the range of electronics. Engineers must make allowances for this unusual behaviour in their designs. The physics is quite rich in principle; simple commensuration might give way to chaotic response in some circumstances. □

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Industrial fruit

FRUIT, that essential part of every healthy diet, is the expensive product of an inefficient process. A fruit tree occupies lots of space, takes years to grow, and yields annually a tiny fraction of its weight as fruit, which must then be harvested by hand. Daedalus reckons there has to be a better way.

Plants, he points out, have a remarkably tolerant immune system. It is quite easy to graft a branch from one species of tree onto the stem of another. All that the branch receives from the stem is sap. So DREADCO horticulturalists are now grafting apple branches, plum branches, and so on, not onto other trees, but onto pipes carrying a synthetic sap under pressure. Provided the sap has the right composition, each branch will bear fruit exactly as if it were attached to a tree.

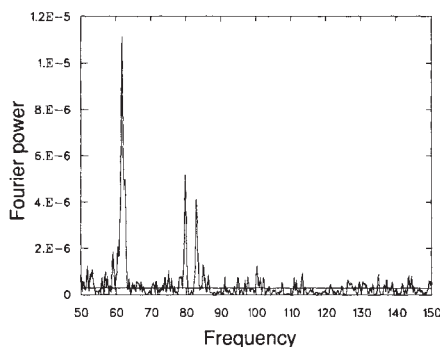
And this is DREADCO's new "Battery Fruit" project. Close-packed rows of fruit-bearing branches, kept at the optimum temperature and illumination in a big windowless building, will be pressurized with synthetic sap from a central source. When ripe, the fruits will be detached mechanically, or possibly chemically by the sudden injection of a shedding-hormone into the sap. A conveyor belt will remove them to the packing station, while the bare branch receives in its sap the correct hormonal signal to start bearing new fruit. Some fruits, like strawberries, will grow without a previous pollination step: but where pollen is needed it will be supplied mechanically. Thus the traditional orchard, with its vast cost in space and time and labour, will be eliminated. Many harvests a year will be obtained. Fruit will be grown intensively and continuously at last.

Organic-husbandry enthusiasts will doubtless denounce battery fruit, like battery chicken, as drab, tasteless and nutritionally impoverished. In fact, it will be extremely healthy. By adding vitamins and minerals to the circulating sap, Daedalus will augment the nutritive value of the final fruit. Even more cunning, he will flavour and perfume the sap with wonderful tastes and smells. Not only will the traditional fruits come from the battery tastier and more appealing than ever; amazing new taste sensations will be possible. A true toffee-apple, a genuine chocolate orange, a tomato tasting of tomato ketchup, will be feasible at last. Bananas redolent of cheese-and-onion, pears made teasingly aromatic with smokey bacon, the pizza-peach, the sausage-strawberry and the hamburger-apple, all will invade the supermarkets. Their flavours will tempt even junk-food addicts, those nutritionally lost souls, into healthier eating.

DAVID JONES

Muonium in fullerite

SIR — We wish to report the observation of muonium after implantation of positive muons into C_{60}/C_{70} fullerite¹. Muonium (Mu ; μ^+e^-) is a light analogue of the hydrogen atom, and has been used to provide information on defect centres in semiconductors²⁻⁴ and radicals in organic compounds^{5,6}. In graphite, only the 'bare' μ^+ signal is observed, whereas in diamond and other semiconductors two forms of muonium ('vacuum' Mu and 'anomalous' Mu^*) occur^{3,4}. We have found that at least 30 per cent of the muons implanted into



Muon-spin-rotation spectrum of C_{60}/C_{70} fullerite at 210 K and an applied field of 58.4 Oe.

fullerite form vacuum Mu and a muonated radical species.

Our experiment, which used standard muon-spin-rotation/relaxation (μSR) techniques, was carried out at the TRIUMF cyclotron, Vancouver. The powder sample of fullerite (C_{60} with about 10 per cent C_{70}) was from MER Corp., Tucson, Arizona. We measured the amplitude and Larmor precession frequencies of μ^+ and Mu signals, over 4 μs after implantation of the muons (capture occurred in less than a nanosecond). The figure shows the main muonium lines obtained at 210 K in an applied field of 58.4 oersted (Oe). The doublet centred at 81.2 MHz is characteristic of vacuum Mu as its splitting corresponds to a μ^+e^- hyperfine interaction of 4,265 MHz, slightly reduced by the medium from the 4,463 MHz for isolated muonium in vacuum²⁻⁵. Such muonium atoms are usually located in interstitial cavities in a variety of solids (including ice), and can be highly mobile. The

stronger signal at 61.7 MHz was assigned to the formation of radicals on the surface. A partner signal at 264 MHz (not shown) implies that this radical has a hyperfine splitting of 325.7 MHz. The radical appears to be isotropic, but it is still to be determined whether this is intrinsic or due to rapid molecular rotation (that is, the radical signal is also a probe of rotational dynamics)^{4,6}. The diamagnetic μ^+ signal at 0.791 MHz accounts for 60 per cent of the total amplitude. No other Mu signals were observed above the 2×10^{-6} level up to 300 MHz. These assignments were confirmed by spectra at two other values of applied field.

The vacuum Mu species might reside inside the C_{60} cages, and/or be almost free at or near the surface of the molecules, where it might react to form the observed radical. It will be interesting to

see whether this Mu can be released into the vacuum, as is the case for very fine silica powders². If ^{13}C is substituted into the fullerene molecules, the muonated radical signal could be used to probe the electron spin density and coupling constants in the carbon rings. As in silica⁷⁻⁹, Mu could react with and provide information about other molecules adsorbed on the molecular surfaces, and so give insight into the possible catalytic properties of fullerite.

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Imprints on DNA fingerprints

SIR — Each autoradiogram in the DNA population databases maintained by the FBI contains a replication of one set of bands from a constant, known, human DNA sample. Comparing the band-length estimates of this control sample made by five laboratory technicians, I found there were statistically significant differences between mean estimates and statistically significant correlations between estimates of the two alleles comprising a pair of bands. These findings have serious implications for forensic DNA fingerprinting.

FBI population databases are separated racially, but the control sample on each autoradiogram is always the same. Therefore, I ignored the database racial designations. The control sample produces two bands with three probes and one band with one probe. The results are shown in the table.

Technician G.S.R.'s estimated band lengths are statistically significantly smaller than those of A.M.G. and L.A.W. Furthermore, the mean band-length estimates by G.S.R. are smaller than those by the other four technicians in 25 of the 28 possible comparisons with G.S.R. Also, H.L.L. estimates the length of the D17S79 band to be statistically significantly larger than do each of the other

four technicians.

It can also be seen in the table that the length estimates of a pair of bands are correlated with each other for each of the technicians. Additionally, partial correlation coefficients of band 1 and band 2 length estimates were calculated for each probe by controlling for technicians, and also for each technician by

PROBE	OBSERVATIONS	NO. OBS.	BAND 1		BAND 2		COVARIANCE	CORR. COEFF.	PROBABILITY
			MEAN	STD. D.	MEAN	STD. D.			
D4S139	GSR	27	9070.63	85.46	7633.67	50.14	2495.22	.5824	.001
	AMG	109	9117.57	91.64	7682.33	69.64	4353.18	.6819	.000
	LAW	25	9136.12	86.28	7689.88	67.86	3475.18	.5935	.002
	HLL	10	9130.50	51.01	7654.50	52.51	1655.72	.6181	.057
	MEC	24	9077.92	118.59	7707.42	96.72	4030.73	.3514	.092
D1S7	GSR	28	12538.75	165.42	9209.82	71.82	6685.44	.5628	.002
	AMG	115	12891.77	272.24	9275.98	90.51	12440.86	.5049	.000
	LAW	17	12716.18	235.06	9252.88	74.46	9308.40	.5318	.028
	HLL	8	12537.38	197.17	9226.00	89.51	16622.14	.9418	.000
	MEC	17	12904.41	326.99	9264.53	100.40	21563.96	.6568	.004
D2S44	GSR	30	3761.23	20.20	2915.20	15.95	95.33	.2960	.112
	AMG	103	3777.87	22.16	2925.08	15.08	181.78	.5440	.000
	LAW	59	3772.69	23.24	2924.49	16.91	244.08	.6209	.000
	HLL	11	3762.00	18.94	2922.73	11.73	58.30	.2623	.436
	MEC	24	3769.12	27.72	2918.50	15.57	309.07	.7162	.000
D17S79	GSR	23	1358.22	12.22					
	AMG	114	1360.26	12.51					
	LAW	62	1363.23	10.39					
	HLL	10	1370.60	7.97					
	MEC	20	1356.30	11.98					

Results of the analysis of band-length estimates from the control lanes on the autoradiograms from which the FBI population databases are constructed, separated by the four probes with which data were generated. The probes are D4S139, D1S7, D2S44 and D17S79, respectively. The results are presented separately for each of the five technicians (observers). The number of observations denotes the number of control lanes in the databases for each technician with each probe. The mean and standard deviation of each band-length estimate are shown. The estimated band lengths are expressed in kilo basepair units. The covariance and the correlation coefficient (r) between the two bands in the control lane are shown, as well as the probability of obtaining that correlation coefficient by chance if the band length estimates were unrelated to each other. Probe D17S79 generates only one detectable band.

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controlling for probes. The partial correlation coefficients are 0.5879, 0.5418 and 0.5482 for probes D4S139, D1S7 and D2S44, respectively. The partial correlation coefficients are 0.5557, 0.5109, 0.5072, 0.8404 and 0.5024 for G.S.R., A.M.G., L.A.W., H.L.L. and M.E.C., respectively. All the partial correlations have chance probabilities less than 0.001.

In the control sample, the two bands are *a priori* independent of each other. Before laboratory treatment, each band is just a constant from a constant sample. Nonetheless, it is clear from these results that the process by which the band length estimations are carried out destroys whatever independence exists between the two alleles of a pair of bands.

These findings have serious practical

implications for the use of these databases to calculate population probabilities for forensic samples: (1) Band-length estimates by different people may not be comparable. (2) The joint probability of obtaining a particular pair of band lengths within a sample is not equal to twice the product of the individual band-length probabilities. (3) A product rule for calculating probabilities across probes also fails because of the pattern of differences between technicians across probes. (4) The disproportional representation of technicians in the racial databases for each probe exacerbates these problems.

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More natural classification

SIR — Woese *et al.*¹, who criticised my proposal for the classification of the living world², miss the basic issue. They fail to realize that there are two ways by which organisms can be ordered. One is the traditional system of classification used almost universally since Linnaeus by botanists and zoologists, in which organisms are grouped in classes on the basis of similarity and relationship. The other is Hennig's "general reference system", in which organisms are grouped strictly phylogenetically, that is according to what 'clade' they belong to³. The arrangement adopted by Woese *et al.* is Hennig's phylogenetic reference system; the classification supported by me is based on the traditional principles of classification, which biology shares with all fields in which items are classified, as are books in a library or goods in a warehouse.

Both groups of prokaryotes, the eubacteria and the archaeobacteria, are very ancient and branched from each other before the origin of the eukaryotes. There is no argument between myself and Woese *et al.* as far as the facts are concerned. The argument is whether a hennigian reference system (containing only branching information) or a traditional classification is the more useful.

I opt for the latter for a number of reasons, some of which I have already stated⁴. A hierarchy of categorical ranks in a traditional classification expresses degrees of difference. To give each of the two subdivisions of the prokaryotes the same rank (domain) as the eukaryotes, which differ by the possession of a nucleus, cytoplasmic organelles and many other drastic characteristics, violates all principles of hierarchical classification. Indeed the two groups of prokaryotes are so similar that the dis-

tinctness of the archaeobacteria was discovered by Woese as recently as 25 years ago. Archaeobacteria and eubacteria are indeed so similar that the definition of bacteria found in the older textbooks of bacteriology fits both. I find it incomprehensible that Woese *et al.* can call a classification artificial which recognizes the drastic difference between prokaryotes and eukaryotes.

Woese *et al.* also violate another basic principle of classification, that the best scheme should be based on as many diagnostic differences as possible. Woese and other authors interested in the branching sequence have, however, limited themselves to a consideration of those few characters that had already branched among the prokaryotes. This ignores the vast majority of eukaryote characteristics and cannot help but result in an unbalanced consideration. A more detailed analysis of the many weaknesses of phylogenetic reference systems has been given elsewhere⁴.

Traditional classifications and hennigian phylogenetic reference systems have very different objectives and are, therefore, not alternatives. If one wants merely to document branching points in the phylogeny, one will use a hennigian cladogram. If one wants to express degrees of difference and more than merely linear relationship, one should adopt a traditional classification.

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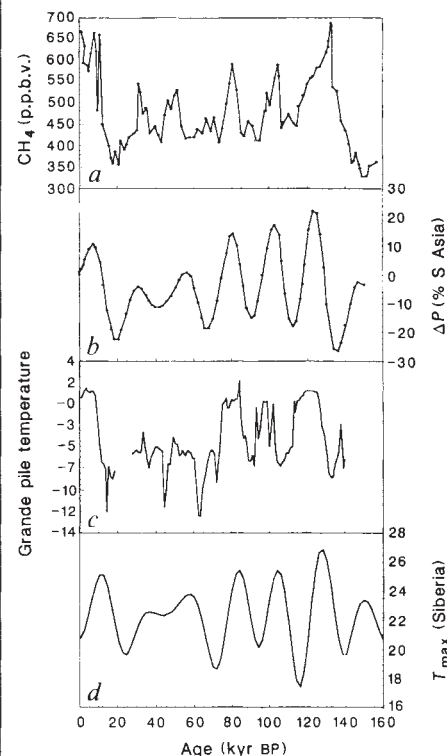
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Ice-age methane variations

SIR — Chappellaz *et al.*¹ note that methane fluctuations in the last glacial cycle, which have a strong 23,000-year (23-kyr) precession component (*a* in the figure), compare favourably with calculated precipitation variations² in the area of the Asian monsoon (*b*). A further justification for the low-latitude origin was the observation that the high-latitude ice volume signal is dominated by 100-kyr oscillations³.



Comparison of methane¹, southern Asian precipitation index², a northeast France (47°N) pollen-temperature record^{5,10}, and central Eurasian (60°N, 100°E) temperature index⁸. The last calculation used a two-dimension energy balance model to examine the filtering effect of geography on orbital forcing. Previous comparisons of the model to general circulation models indicate that it has a sensitivity comparable to general circulation models for seasonal changes over land areas^{11,12}.

Although 23-kyr fluctuations are commonly associated with low-latitude phenomenon, 23-kyr forcing also occurs in high latitudes (see, for example, ref. 4). A pollen record from northeast France (47° N) has significant 23-kyr power (refs 5, 6; *c* in the figure). Further east in Siberia, in the largely ice-free wetlands region that now stores a significant quantity of methane⁷, models of climate⁸ indicate summer temperature variations at 60° N of about 10 °C (*d*) that have a strong 23-kyr component. In fact, the largest modelled temperature

response on Earth occurs in this region, because the huge Eurasian landmass is very sensitive to seasonal changes in precession forcing.

A comparison (*b* and *d*) of modelled low-latitude and high-latitude responses over the last glacial cycle indicates a great deal of similarity. The precipitation curve is phase-shifted with respect to temperature, because the effect of ice sheets has been included in the former² but not the latter. Even though the simulated temperature curve is for a largely ice-free region⁸, timing of temperature variations could also be shifted somewhat in this area as a result of downwind influence from ice sheets.

It therefore seems that northern wetlands could also be the source of changes in methane during the ice age. It may not be possible to use climate models to discriminate between low- and high-latitude methane sources, but methane isotope studies may in future shed light on the problem, as low- and high-latitude wetland methane sources differ

in their carbon-isotope signatures by about 12 parts per thousand (for example, ref. 9).

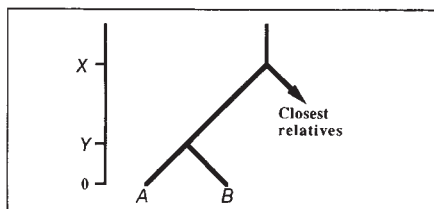
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Bird community structure

SIR — A recent paper from our research group¹ pointed out that the correlation between abundance and body size among species within tribes of British birds is frequently positive, particularly when a tribe has no close phylogenetic relatives in Britain. Our investigations of a similar pattern occurring in local, single-habitat communities suggest that the date at which a tribe radiated, rather than the



A tribe of birds consisting of two extant species (*A* and *B*) shared their most recent common ancestor with each other *Y* time units ago and their most recent common ancestor with other birds *X* time units ago. The measure *Y* is a better predictor than is *X* of the correlation between population density and body size within tribes.

date at which it originated, is a better predictor of the correlation between abundance and body size. In other words, the date *X* in the figure may have been a surrogate for date *Y* (or some measure closely correlated with *Y*). Any explanation of the original result will need to take account of this finding.

We have examined data from 90 bird communities sampled from small areas (10–100 ha) of defined habitat throughout the world². Nee *et al.* used a measure of the time at which members of a tribe last shared a common ancestor with

members of other tribes: the date of origin of the tribe (*X*). We have also obtained an estimate of the time when all members of a tribe last shared a common ancestor with each other: the date of radiation of the tribe (*Y*) (ref. 4). We normalized all data using Table 20 of ref. 3. Those tribes which have no close relatives in the world (those for which *X* is large) tend to show positive body-size abundance relationships more frequently than those with close relatives ($r=0.36$, $n=30$, $P=0.05$). This is also true of tribes which radiated later (those for which *Y* is large) ($r=0.61$, $n=30$, $P=0.0003$).

When we control for the date of radiation (*Y*) in a partial correlation, the date of origin (*X*) is not a significant predictor of how frequently a tribe of birds shows a positive relationship between abundance and body size ($r=0.10$, $n=30$, $P=0.56$). However, when we control for the date of origin, the date of radiation is a significant predictor of the frequency with which the relationship between abundance and body size is positive ($r=0.54$, $n=30$, $P=0.002$). We find similar results with the British bird data used by Nee *et al.*, who suggested that the date of origin of a tribe may relate to

ecological guild structure. Whether the date of radiation might be interpreted in like manner remains a subject for investigation. We shall describe elsewhere how body-size abundance patterns differ when we compare (1) a tribe's relatedness to the other tribes in its community rather than to other tribes in the world; and (2) single-habitat communities with geopolitical regions, such as Britain or Sweden.

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Iron still comes from above

SIR — Sunda *et al.*¹ report that open ocean phytoplankton require very little iron indeed; less than 10% of the minimum amounts thought necessary to meet the metabolic needs of plant cells for growth based on iron enzymatic requirements for photosynthesis, respiration and NO₃ reduction². They suggest that we should reassess our conclusion that amounts of dissolved iron in open ocean upwelling water are too low, and that this essential element must be supplied from atmospheric deposition.

We recently measured³ dissolved iron in the equatorial Pacific (0°; 140° W), and we were unable to detect (< 0.02 nmol kg⁻¹) this essential element at 60 m, the depth of maximum upwelling⁴. Assuming that the iron concentration is about 0.01 nmol kg⁻¹, this would be enough to permit the phytoplankton to produce only 5 μmol carbon based on the very high 500,000 C/1 Fe ratio reported by Sunda *et al.*¹. On the other hand, the NO₃ at 60m, 8.1 μmol kg⁻¹, would support the production of 20 times more carbon based on the redfieldian ratio of 6.6 C/1N. The situation is worse south of the Equator (3°S; 140° W). Here, dissolved Fe concentrations do not begin to increase systematically beyond 0.04 nmol kg⁻¹ until a depth of 150 m is exceeded. I still believe that open ocean 'diatomic' iron comes from above, not from below. In areas like the equatorial Pacific, where little or none comes from above, a deficiency of this element limits phytoplankton growth.

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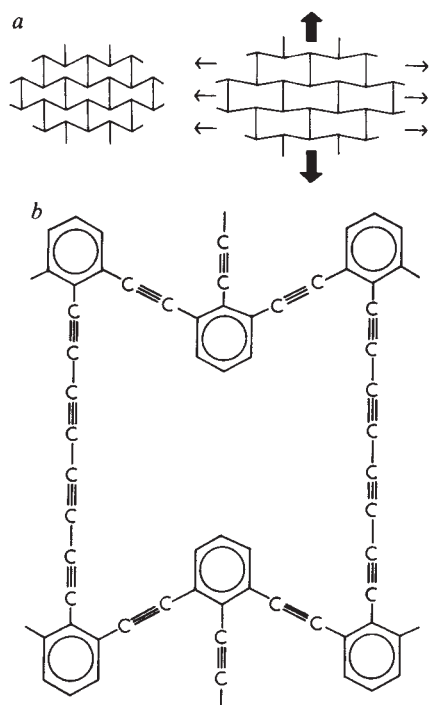
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Molecular network design

SIR — Naturally occurring materials have a positive Poisson's ratio¹; when stretched, the material becomes thinner. Recently, synthetic materials have been processed that exhibit a negative Poisson's ratio, (becoming fatter when stretched)²⁻⁶. Such materials can have improved mechanical properties, such as enhanced shear moduli, indentation resistance and fracture toughness^{2,7}. All these materials exhibit a negative Poisson's ratio as a result of microstructures, or geometric units, that are at least tens of micrometres in size. We have used molecular modelling techniques to design a molecular network with a negative Poisson's ratio. By altering the geometry of the repeat unit, Poisson's ratio can be varied to produce positive or negative values with mechanical properties that are either isotropic or anisotropic.

The Poisson's ratio of a material is given by $\nu_{xy} = -\epsilon_y/\epsilon_x$, where ϵ_x is an applied tensile strain and ϵ_y is the resulting tensile strain in the transverse direction. For isotropic materials $\nu_{xy} = \nu_{yx} = \nu$ with the limits $-1 < \nu < 1/2$. For anisotropic materials neither of these limits apply. For example, orthotropic materials may have Poisson's ratios anywhere within the range $|\nu_{xy}| < (E_x/E_y)^{1/2}$, where E_x and E_y are Young's moduli in the orthogonal directions x and y ^{8,9}.

Examples of materials that have been fabricated with a negative Poisson's ratio



a, Two-dimensional re-entrant honeycomb, showing transverse expansion on stretching. b, (1,4)-reflexyne with a negative Poisson's ratio.

are two-dimensional honeycombs⁴, three-dimensional foams^{2,3} and microporous polymers^{5,6,10}. An example of the simplest microstructure that produces this effect is illustrated schematically in Fig. a. A conventional hexagonal honeycomb has a positive Poisson's ratio, but making the cells 're-entrant', as in the figure, produces a negative Poisson's ratio.

The honeycombs and foams manufactured so far have low densities and are intrinsically weak and flexible. By reproducing this geometric unit on the molecular scale, however, it should be possible to take advantage of the innate free volume found, for example, in polymeric structures to increase the density and absolute stiffness of the material. Figure b illustrates a molecular network structure that reproduces, on the molecular scale, the features of the geometry in Fig. a. We suggest non-systematic naming convention for the hexagonal network of the form (n,m) -reflexyne for the honeycomb structure, where n is the number of acetylene links on the diagonal branches and m the number of links on the vertical branches, and (n,m) -reflexyne for the re-entrant structure. By altering the values of n and m , the structure can be either isotropic or anisotropic with varying Poisson's ratios. The use of benzene rings at the junctions and acetylene groups along the branches creates, in principle, a planar two-dimensional structure. Although such a network may be hard to synthesize, it provides a simple example for molecular tailoring of Poisson's ratio.

A model has been developed¹¹ that successfully predicts Poisson's ratio for macroscopic honeycombs. This model assumes that deformation occurs by flexure, with no stretching of the cell arms, whereas in the molecular structure of Fig. b deformation will occur by flexing, stretching and rotation of the arms. We have used a molecular-mechanics program¹² which incorporates a standard valence force field to model the deformation of this structure and thus to calculate Poisson's ratio. Because of the symmetry of the network, only one unit cell need be modelled to determine the network's deformation behaviour. A force is applied in the x direction and the resulting displacements in the x and y directions are used to obtain ν_{xy} . Similarly, a force in the y direction is used to obtain ν_{yx} . Strains of less than 5% are used to ensure elastic behaviour.

In the table we give values for five (n,m) -reflexyne networks. The re-entrant structure does indeed produce negative Poisson's ratios, although in

general the simple flexure model overestimates Poisson's ratio relative to the molecular-mechanics approach. This is because both stretching and rotational

CALCULATED VALUES OF POISSON'S RATIOS FOR (n,m) -REFLEXYNE

n	m	Flexure model			Molecular mechanics		
		ν_{xy}	ν_{yx}	ν_{xy}/ν_{yx}	ν_{xy}	ν_{yx}	ν_{xy}/ν_{yx}
2	6	-0.96	-1.04	0.93	-0.84	-0.81	1.04
2	8	-0.95	-1.05	0.92	-0.62	-1.14	0.55
1	4	-0.93	-1.08	0.86	-0.59	-0.83	0.72
1	6	-0.63	-1.58	0.40	-0.43	-1.14	0.38
1	8	-0.51	-1.96	0.26	-0.31	-1.47	0.21

deformation will tend to increase the longitudinal deformation at the expense of the transverse. The value of Poisson's ratio can be altered by changing the shape of the repeat unit. $\nu_{xy} = 1$ represents a truly isotropic structure. $\nu_{xy} = -1$ is only quasi-isotropic in that Poisson's ratios measured by stretching along directions away from the principal axes will have different values; it is a square-symmetric structure.

The molecular network described here represents a first attempt at designing a material that demonstrates a negative Poisson's ratio owing to mechanisms acting at the molecular level. We are now investigating more complex structures that are more amenable to synthesis that have been proposed by G. Wei and S. F. Edwards of Cambridge University. To avoid the cumbersome phrase 'negative-Poisson's-ratio materials', we suggest that they be called auxetic materials, or auxetics (from the Greek *auxetos*: that may be increased, referring to the width and volume increase when stretched).

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Crusades and rackets

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Vitamin C and Cancer: Medicine or Politics. By Evelleen Richards. *St Martin's Press/Macmillan*: 1991. Pp. 269. \$35, £35.

I HAVE spoken to Linus Pauling just once — in the mid-1970s when I was secretary of the Medical Research Council's Cancer Therapy Committee. His son Peter, whom I knew slightly from a chance meeting years before, used me to ask the committee if they would be prepared to carry out a trial of vitamin C as a treatment for cancer. One afternoon I answered the phone and a voice said, "My name is Linus Pauling, I think you know my son." It was a disarming opening. But it was going to take more than that to get what he wanted.

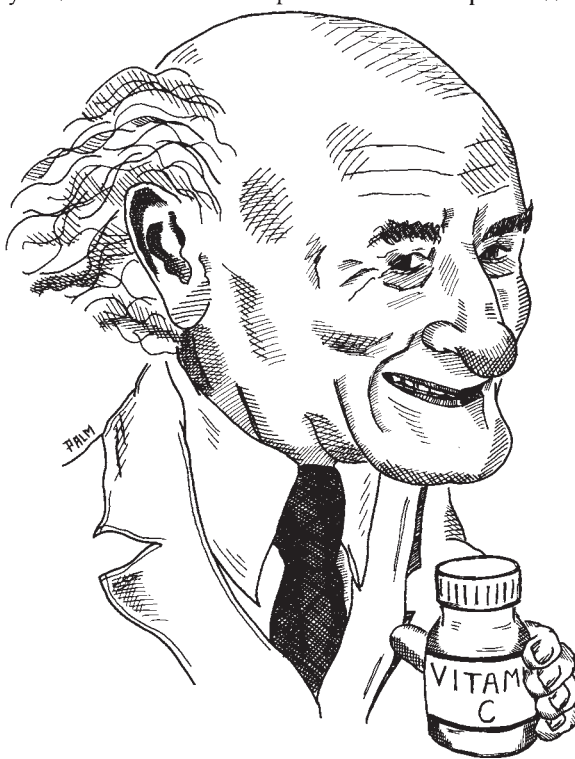
In the event the committee decided against a trial, seeing no particularly compelling reason to do one. Pauling must have been disappointed. I know I was, feeling that if Pauling thought it was a good idea then it probably was. And, having spent some years working on collagen and being interested in its crosslinking, it seemed there was enough of a possible mechanism by which vitamin C might inhibit the spread of cancer — by toughening up the connective tissue — to warrant a trial.

That episode represented, in miniature, the struggle that has now engaged Pauling for more than 20 years. Obsessed by his belief that vitamin C is good for cancer patients, he has spent two decades trying to persuade what he sees as the American medical establishment to take his belief seriously. For their part, judging by Richards' book, they believe they have, having weighed his idea in the balance of the randomized clinical trial but found it wanting.

At one level, Richards' book is a straightforward piece of historical narrative, telling it how it was, and is, because the story is presumably not over; from Pauling's early contact with Ewan Cameron, a Scottish surgeon who triggered the whole thing, through to and beyond the clinical trials carried out by the Mayo Clinic with their conclusion that there was nothing in the idea. This conclusion was followed by an avalanche of acrimony — of accusations and counter-accusations of sharp practice, unprofessional conduct, of bringing science and medicine into disrepute, and of threats of litigation. As Richards cheer-

fully says, it is a story that has everything. Well, nearly everything. It doesn't seem to have an ending. Is Vitamin C good for cancer patients or not? But, of course, that does not really matter because Richards is simply using the saga to take the lid off medicine and medical science.

She tries — and with considerable success — to give us insights into how new ideas are accepted and become part



of the medicine of the day, or aren't and don't. And because the two Mayo Clinic trials figured so largely in generating and sustaining the particular drama, Richards hooks her arguments onto the idea of the clinical trial put forward as the paradigm for how these choices are made. It is a well researched book as far as I can judge, and a well written one, and it addresses an intriguing and complicated question.

But I could not help thinking that it missed a point. The history of randomized clinical trials is not really a history of established medical practice setting itself up with this particular methodology to exclude outsiders. On the contrary, a great deal of the struggle surrounding trials has been between statisticians who saw potential value in randomized trials and doctors who often did not, or who

simply misunderstood them, even seeing them as an intrusion into the way they practised. There is an issue central to medicine here: the tension between a doctor practising on the basis of his or her own personal clinical experience, and practising on the basis of someone else's, which they can be reluctant to do. The irony running through the book is that the medical establishment were behaving more like statisticians whereas Pauling was behaving more like a traditional doctor.

What seems to matter most in clinical-trial methodology is that there is at the outset real interest by doctors in the question that is being addressed. This ensures that the trial is large enough to provide an answer sufficiently clear and to convince both them and others of its value. Richards makes an interesting story in comparing inconsistent attitudes to trials of vitamin C and the anticancer drug 5-fluorouracil. But she could have looked at other examples. Massive clinical trials have shown convincingly that aspirin is of value in warding off strokes, heart attacks and pregnancy-induced high blood pressure, and also that its use is often preferable to much more expensive and well publicized drugs such as streptokinase. It has not been so difficult to persuade large numbers of doctors to join in these enterprises and come up with an answer they take seriously — and act on.

There is at least some evidence that a lot of publicity tends to accompany only those trials involving a clash of what are represented as different medical cultures. It is worth noting that when it was begun, the Medical Research Council's trial of vitamins to prevent spina bifida aroused considerable passion. Its recently published finding that folic acid would prevent the majority of cases has passed fairly quietly. Would this have been the case had the trial come down against the compound?

Richards is at pains to tell us that however good a drama the story provides, she is really interested in what it tells us about medicine. But the fact remains that she has chosen an extreme case to make her point because she believes it makes it most clearly. And it is a very good story. □

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■ Just published by the Royal Society of Chemistry is *Vitamin C: Its Chemistry and Biochemistry* by Michael B. Davis, John Austin and David A. Partridge. Price is £13.50 (pbk). □

Reasons to be cheerful

D. J. Drewry

The Antarctic Treaty System in World Politics. Edited by Arnfinn Jorgansen-Dahl and Willy Ostreng. *Macmillan in association with the Fridtjof Nansen Institute*. 1991. Pp. 475. £45, \$79.95.

THE heightened interest in Antarctica over recent years stems mainly from its value as a large, climatically extreme region where scientific research of global relevance can be undertaken. For here has been observed the worsening of the springtime depletion in stratospheric ozone; an archive of palaeoclimate in ice cores; and the response of ice sheets to global warming with consequent change in sea levels. But another reason for curiosity in the area is the remarkable political framework provided by the Antarctic Treaty, which has sustained Antarctica as the only continent free of conflict and confrontation over the last 30 years. Moreover, the treaty has become an evolutionary 'system' of international governance, regulatory measures (recommendations, conventions and protocols) having been successively applied to new areas of concern. These measures have been coupled to policies of intergovernmental bodies (for example, the World Meteorological Organization), the Scientific Committee on Antarctic Research (for international co-operation in Antarctic science) and, more recently, the Council of Managers of National Antarctic Programmes.

The treaty aims to maintain peace in the region and recognizes the contribution that can be made by science. One of its hallmarks is the international collaboration that it fosters. Indeed, for an area almost twice that of Australia (14 million square kilometres) and possessing one of the planet's harshest environments, cooperation is essential. No nation could single-handedly do even a proportion of the relevant and exciting science or confront the logistic challenges offered by Antarctica.

Issues that have come before the Antarctic Treaty System in the last decade have engaged the interest of scientists, managers, diplomats, lawyers and environmentalists, and have often been discussed at important international conferences. One of the most recent of these was held at the Fridtjof Nansen Institute in Oslo, Norway, in May 1990,

and it is the proceedings of this meeting that make up the 25 chapters in this well organized book.

The book starts by looking at the most recent conventions of the system: the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR) adopted in 1980; and the Convention for the Regulation of Antarctic Mineral Resources (CRAMRA), which has not been adopted and indeed has now been supplemented by the September 1991 Madrid Protocol for the Protection of the Antarctic Environment.

Much criticism has been levelled at CCAMLR by those who consider it to be ineffective in controlling over-exploitation, despite its trail-breaking, scientifically based (ecosystem) approach to the management of the Southern

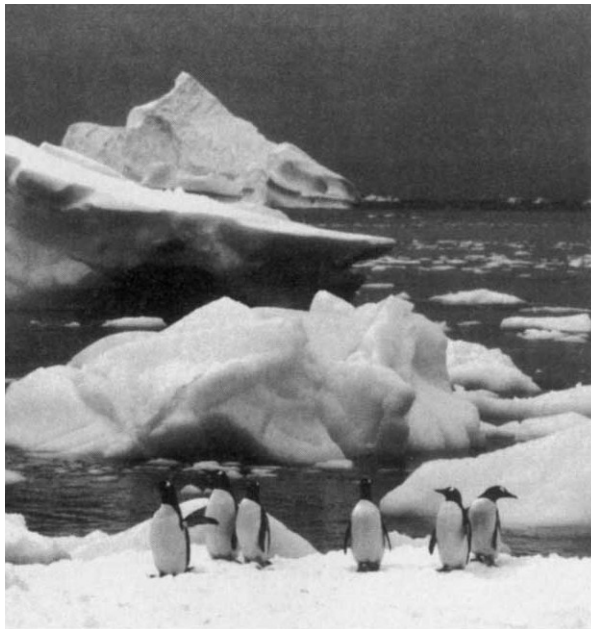
in its negotiation is set out by R. Anderson. L. Kimball argues cogently that, despite the rejection of the convention by Australia and France on account of domestic political expediency (the case is elaborated by A. Brown), its provisions may ironically prove to have been the best for protecting Antarctica, certainly better than those of the Madrid protocol.

This view is also explored by C. Joyner in the next section of the book, in which the issues of resource management and environmental protection are put into a wider context. The alternatives to CRAMRA that have emerged from the debate about its political acceptability are based on the idea of a 'world park' and a system for the protection of a range of different kinds of environments. In detailing these alternatives, J.

Barnes discusses the role being played by nongovernmental organizations, such as Greenpeace, Friends of the Earth, and the Antarctic and Southern Ocean Coalition. The weakness of his argument lies in his scientific justification for reducing, controlling or banning human activity. Here he overestimates the likely environmental impact and extrapolates speciously. This detracts from an otherwise genuinely useful perspective. Aspects other than exploitation in Antarctica are also reviewed; these are principally matters of science (C. Rinaldi and A. Karlqvist) and sovereignty (P. Beck).

There then follows an assessment of the status of the system within the world community. An important matter exercising the collective mind of the treaty signatories since 1983 has been the relationship of the system with the United Nations. The treaty

embraces the furtherance of the purposes and principles embodied in the charter of the United Nations through pursuit of peaceful activities and international harmony, yet has resisted pressures from the United Nations to be more active and interventionist (S. Harris). The campaign for raising the 'question of Antarctica' within the general assembly of the United Nations, extending the principle of the "common heritage of mankind" to Antarctica and challenging the political make-up of the system, has been led by the Malaysian government. M. Haron briefly reviews the key arguments behind this campaign and speculates on the likelihood of the system's succumbing to these external pressures. A. Bos and V. Golytsin deal with the status and legitimacy of the system in international law, and its relationships with the 1982 Law of the Sea Conven-



Cold continent — safe from exploitation and conflict? Picture taken from *Wild Ice* by R. Naveen et al., (Smithsonian Institution Press, \$129).

Ocean fishery (as explained by M. Basen and J. Beddington). O. Vicuna and J.-P. Puissochet make critical and, at times, opposed, assessments of the performance of the convention, focusing on the problems surrounding the consensus principle (by which all parties, not a majority, must agree to recommendations) and the role of the scientific committee. J. Heap, doyen of the treaty consultative parties, provides a stimulating critique of the system, emphasizing the improvement, albeit slow, in the effectiveness of the convention's regulatory mechanisms.

If CCAMLR has so far been the Cinderella of the system, then CRAMRA is its ugly sister. Conceived a decade ago, the precepts and political climate changed dramatically as the convention moved towards adoption in 1988: the fascinating chronology of compromises

tion, where the Southern Ocean, its exploitation and its environmental protection, provide an intriguing area of legal overlap (V. Safronchuk).

The future is the territory of the final section of the book. There is little doubt that the system has gained considerable momentum since the treaty came into force in 1961. It has adapted to new needs, typically political, more recently environmental. The thirtieth anniversary of the signing of the treaty passed on 23 June 1991, since when it has been possible for consultative parties to seek overall review according to the treaty's initial provisions. With 186 wide-ranging recommendations already agreed at the biennial consultative meetings, the treaty is not ossified or stranded in the era of the Cold War or the International Geophysical Year, but is still contemporary and relevant. As outlined by O. Stokke, the system is a useful model for international cooperation in a wider context. Certainly it has demonstrated an

ability to survive and contribute to change. Yet problems remain. K. Meser and R. Breth tackle the issue of the need for stronger institutionalization through the creation of a permanent secretariat, a *cause célèbre* that has dogged the system for many years.

Another detail requiring urgent attention is how to regulate tourism, which is having increasingly detrimental effects on the environment. The sinking of the *Bahia Paraíso* in 1989 off Palmer Station, where 81 tourists on board had visited US scientists, and the subsequent concern about oil pollution, were the direct result of mixing tourism with the resupply of government research stations. I. Nicholson reviews this area, discussing jurisdiction, legal arrangements, and environmental and safety problems. Finally, both R. Falk and W. Ostreng examine unresolved conflicts of interest within the system, as well as its long-term viability and possible alternatives to its procedures.

It is fitting to conclude that throughout the book the contributors recognize the outstanding success of the Antarctic Treaty System in keeping the peace, shielding crucial scientific research from erosion or manipulation, embracing political change and stimulating international cooperation. For those seeking a broad review of contemporary Antarctic affairs, the volume is thoroughly recommended. □

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■ In a similar vein is *Antarctica and Global Climatic Change* edited by Colin Harris and Bernard Stonehouse, a volume arising from a 1990 British/New Zealand symposium. The book has 15 contributors, including D. J. Drewry, and is divided into three main sections: current state of knowledge; atmosphere, ice and ocean; and ecology and management responses. Published by Belhaven Press, price is £33. □

Dangerous liaisons

Robert L. Wesson

Earthquake Hazard Analysis: Issues and Insights By Leon Reiter. *Columbia University Press: 1991. Pp. 254. \$75.*

GLOBAL warming, the depletion of the ozone layer, toxic waste disposal, food and drugs, AIDS, aircraft and highways — all offer threats against which the citizens of democracies look to their governments for protection. All involve processes that are incompletely understood. And, commonly, the necessary regulatory decisions must be made by governments in a highly charged economic, political and social atmosphere. In this book, Reiter summarizes and assesses the body of techniques used to analyse the hazards posed by a phenomenon of particular concern — earthquakes. His intended audience ranges from scientists to lawyers, and his vantage point as a senior member of the US Nuclear Regulatory Commission (the body responsible for licensing commercial nuclear power reactors in the United States), together with his keen mind and capacity for independent thought, amply qualify him for the task.

The sensitivity of nuclear reactor sites to strong ground motion means that it is important to consider the possible consequences of earthquakes when designing reactors, even for areas of relatively low earthquake activity. The practice so far in the United States has been to design and license commercial reactors according to the hazard associated with

the site chosen. Regardless of whether this is good public policy, the practice has certainly led to a great deal of debate about the earthquake hazards at many sites. It has also produced a terrific growth in information and methods for analysing and describing the hazards. In his book, Reiter summarizes these in a most even-handed and insightful way.

Reiter takes an understated look at the sociology of the community that has arisen in the United States to deal with these issues: engineers who see themselves as the interpreters of reality, needing answers to solve today's problems; lawyers who see themselves as the resolvers of disputes; and scientists (seismologists and geologists) who see themselves as seekers of truth. As a member of the third cultural group, I must confess to mild horror at seeing our assumptions, compromises, inadequacies and lack of fundamental understanding of earthquakes accurately characterized throughout the book. It is fair, but it hurts. The image arises of an eighteenth-century physician, who although lacking understanding of disease is compelled to do something and so prescribes bleeding. But what to do? Scientists cannot expect society to stop in its tracks until the origins of, say, the 1886 earthquake in Charleston, South Carolina, are satisfactorily resolved. (It may well turn out that our failure to resolve this issue is not in fact the stumbling block to mitigating earthquake hazards on the eastern seaboard of the United States, even though I would argue that it is important.)

How are the nature and magnitude of earthquake hazards best characterized? Reiter lays out the methodologies, both deterministic and probabilistic, that have been developed to tackle the problem.

He also describes their seismological and engineering underpinnings, perhaps a little tediously for specialists, perhaps a little briefly for neophytes, but providing enough by way of an introduction. The many examples and applications as well as dilemmas that he describes in doing so are well chosen. The treatment does, however, require the degree of mathematical ability that is usually associated with a background in physical science or engineering, so it would take an exceptional lawyer to be at home here. But for those with the right credentials, the presentation is sufficiently self-contained for the main ideas to be rapidly grasped.

Specialists will find the book a useful assessment of the state of the art. And for those who have the right background and who are interested in the broader issue of hazard analysis in general, the book should prove to be an illuminating case study of one subdiscipline, and of the interplay between science and engineering. Most important is Reiter's commentary on the relative merits, roles and importance of the various approaches. As he states in conclusion: "Much is known about earthquakes and much is not. Seismic hazard analysis is most successful when it can effectively characterize this state of knowledge in those different ways needed by the society in which we live." □

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■ New from Kluwer is *Seismic Anisotropy in the Earth* by V. Babuska and M. Clara, an introduction for researchers and students in the fields of seismology, tectonophysics and geology. Price is Dfl.100, \$59, £37. □

The makings of a marathon

H. C. Bennet-Clark

Microscopic Anatomy of Invertebrates, Vols 1–3 of 15. Edited by F. W. Harrison and John O. Corliss/Jane A. Westfall/Burton J. Bogitsch. Wiley-Liss/Wiley: 1990/1991. Pp. 493/436/347. Each \$150, £116.50.

THE idea of reviewing the first few volumes of what is intended eventually to be a 15-volume series on invertebrate anatomy is one that would fill most with gloom. Uppermost is the fear of having to wade through endless irrelevant or unintelligible photomicrographs. The difference in this case, though, is that an important and valuable work of scholarship has been achieved.

A work of this type relies on the quality of the material presented, which in turn depends on the strength of the team of authors and the uniformity of overall editorial control. So what is the overall impression? The presentation is attractive, the quality of paper is high, and the reproduction, whether of line drawings or photomicrographs, is clean and detailed: publication expense has not been stinted, and this shows in the mostly excellent illustrations, many of them reproduced here for the first time. The pictures complement a series of useful descriptive texts, each supplemented by extensive bibliographies and taxonomic and subject indices.

What has gone wrong? Happily, fairly little, but I found the layout of the volume on Protozoa confusing and, from chapter to chapter, inconsistent. A contents page for each chapter would have helped me to find my way around. For example, the chapter on Mastigophora starts with a treatment of size, shape and symmetry, whereas that on Ciliophora starts with ecology and moves on to evolution and taxonomy. This would have been fine had I easily been able to locate the taxonomy section for Mastigophora or the microanatomy section for Ciliophora.

And another quibble: although most of the illustrations have either a scale or details of magnification, the microscopic technique used is not always stated. Light microphotographs sometimes jostle promiscuously with transmission and scanning electron micrographs — yes, of course we can usually work out which is which, but for consistency we should be told.

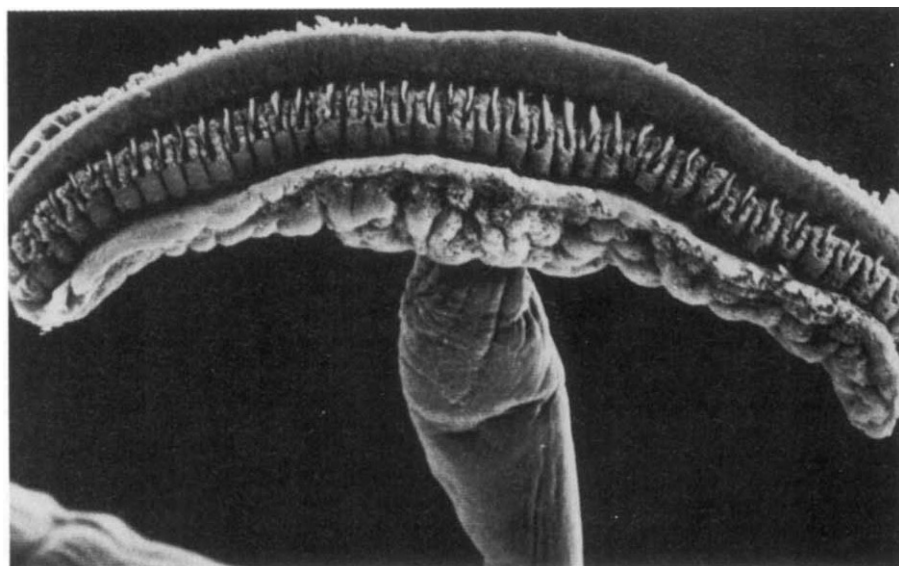
Series of chapters treat the different groups: Protista are covered in four chapters and Cnidaria (polyps, corals, sea-anemones, jellyfish, hydra) in three,

but Porifera (sponges) and Ctenophora (comb-jellies, sea acorns) have chapters to themselves. This division of the taxa into broad systematic units leads to a comparative anatomical survey within each taxon.

The first volume tackles Protozoa, but not in an entirely contemporary way. Indeed, do we still recognize the Protozoa in 1991? In an introductory chapter, J. O. Corliss discusses the problems of splitting or clumping the various taxa of eukaryotic microorganisms. He emerges with a working compromise between the extremes of splitting into more than 20 unlinked phyla and the now-archaic clumping into one phylum with

difficulty with a work such as this, although in this respect the chapter on Hydrozoa is far better.

So on to the third volume: Platyhelminthes (flat worms) and Nemertinea (ribbon worms). Of the Platyhelminthes, free-living turbellarians receive as much treatment as parasitic flukes and tapeworms together, despite the greater importance of the last two classes as the cause of diseases such as schistosomiasis and fascioliasis, as well as hydatid cysts. But much of this apparent imbalance results from having to deal with the problems of the taxonomic diversity of the Turbellaria, leading to a rather confusing text that is light on illustrations.



Gripping stuff — Scanning electron micrograph of the clamp of *Cathetocephalus thatcheri*, a tapeworm that infects the sandbar shark. Magnification, $\times 4,650$.

the 'protozoan' classes of flagellates, amoebae, sporozoans and ciliates. These clumpings are used to divide the volume into chapters, but the 'five kingdoms' phyla are used to divide within the chapters, so one must have a working knowledge of the taxonomy of protists to unravel the differences that appear between the anatomy of the Pymnesiophyta and the Euglenophyta. Fortunately Corliss provides a guide, but it is still not easy to browse.

Far more accessible is the second volume, covering Placozoa, Porifera, Cnidaria and Ctenophora. I particularly commend the exciting treatment of the Hydrozoa that ranges far beyond microanatomy into aspects of morphogenesis, functional morphology and physiology. The volume opens with a brief but useful account of the four phyla and thereafter the chapter-by-chapter treatment is logical and consistent. Although the micrographs and descriptions are excellent, the chapter on Ctenophora is let down by a lack of general or three-dimensional diagrams or small-scale micrographs next to the highly magnified details of anatomy: this is a common

The chapter would have been improved greatly by a lengthier and more descriptive systematic introduction. In the chapter on flukes, there is amazingly no decent illustration of a ciliated first-stage larva (miracidium) of Digenea (liver flukes), even though the anatomy of the final larva (cercaria) and adult are well and diversely illustrated. The high point of the volume has to be the chapter on tapeworms: excellent scanning electron micrographs of attachment mechanisms and of the body surface; admirable diagrams, description and photomicrographs of the body wall; and intimate details of the reproductive system.

What a marathon. The editor is to be congratulated for keeping a fairly tight editorial hand on the enterprise but more for having the idea in the first place: he has succeeded in getting world leaders in each field as his authors and they, in turn, have pulled together an immense and valuable amount of information. □

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Estimation of new production in the ocean by compound remote sensing

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Oceanic new production can be estimated from remotely sensed data on ocean colour and temperature. This approach, which depends on parameterizations developed from ship observations, as well as on satellite data, yields more representative estimates of the large-scale average new production than those calculated from ship data alone.

REMOTE sensing has been used successfully in biological oceanography for measuring properties that have a particular expression in the electromagnetic spectrum. Phytoplankton biomass (chlorophyll concentration) can be indexed by virtue of its effect on ocean colour using the coastal zone colour scanner, CZCS (refs 1, 2); primary production can be estimated through its known dependence on available light and biomass³. But we know of no electromagnetic principle that could be exploited as a basis for direct measurement by remote sensing of new production, that part of primary production dependent on nutrients allochthonous to the photic zone⁴. On the other hand, synoptic mapping of new production is an important require-

ment of global biogeochemical studies⁵ that can be met only through use of remotely sensed data. This suggests a need to explore indirect methods for estimation of new production from satellites, for example through interpretation of the temperature field^{3,5,6}. One such method has been applied to the study of an upwelling regime off the west coast of Africa⁷. We present here a simpler approach to estimation of new production by remote sensing. We illustrate the method 'compound remote sensing', with data for the Georges Bank (Fig. 1) collected during summer, but the approach would be valid for other regions as well.

Method and sources

New production (P_n) is that part of total primary production (P_t) in excess of local community metabolism^{5,8}. The ratio of P_n to P_t is called the f -ratio⁹. Estimation of P_n by compound remote sensing is based on the following sequence of ideas. Sea surface temperature (SST) is accessible to remote sensing through the advanced very-high-resolution radiometer (AVHRR) technique¹⁰. Nitrate concentration (N) can often be estimated from temperature (T), a method that works particularly well on Georges Bank¹¹. In coastal waters especially, f is a well-known^{12,13} function of N . Remotely sensed data on

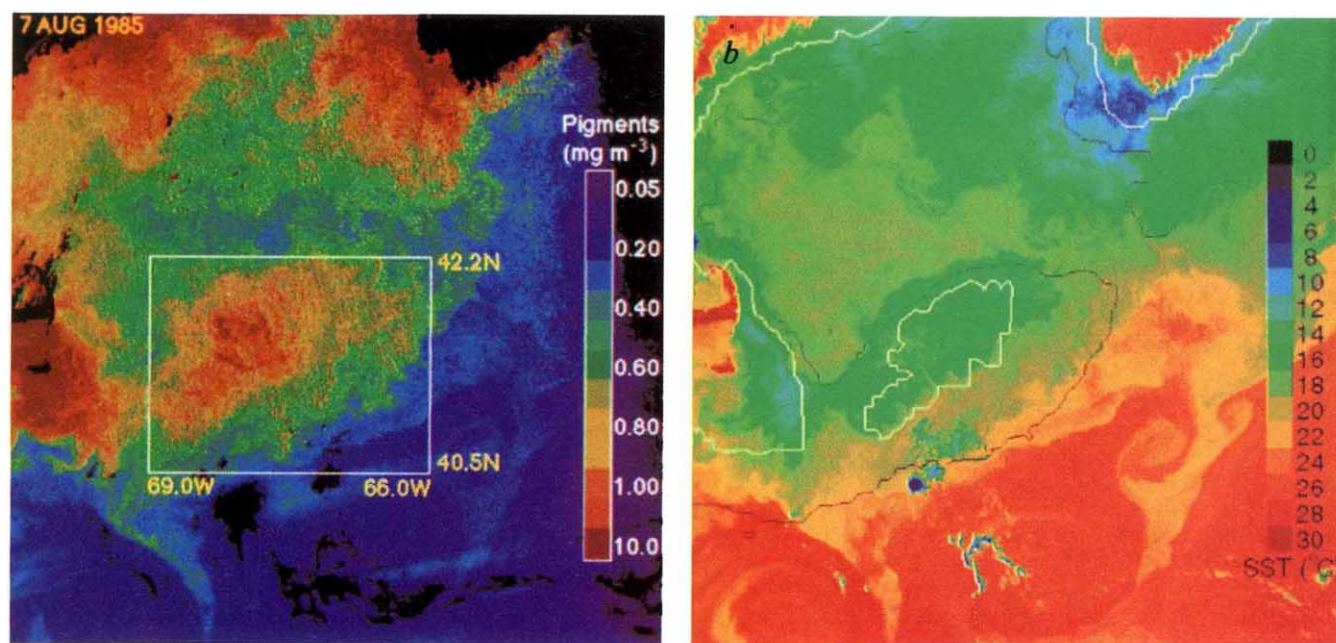


FIG. 1 Satellite images of Georges Bank region for 7 August 1985. The images are mapped on identical projections. Southern Nova Scotia is at the top right-hand side of these images and Cape Cod is at the left centre. Two Gulf stream eddies are visible in the lower portion of the images. *a*, Distribution of pigments from the CZCS. The white box indicates the region for which the primary production calculations were carried out. Georges

Bank, in the middle of the box, shows high biomass compared to the surrounding waters. *b*, Sea surface temperature distribution, derived from AVHRR data. Depth contours of 50 and 100 m are shown in white and black respectively. Georges Bank is cooler and shallower than the surrounding waters. Regions of coastal upwelling are also seen, off Nova Scotia and Cape Cod.

TABLE 1 Estimated values of total and new production, and comparison with *in situ* estimates

Source	P_t^*		$P_n^\dagger$		$f\text{-ratio}^\ddagger$		$B^\S$		$X^\parallel$	$n^\P$	Region
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.			
Satellite data	1,700	950	520	320	0.31	0.08	5.39	1.23	310	2,504	Mixed
1985 cruise data	1,800	—	570	—	0.31		3.85	—	470	1	
O'Reilly <i>et al.</i> ²²	1,500	490								5	
Satellite data	910	430	490	220	0.55	0.06	1.48	0.80	620	5,465	Frontal
1985 cruise data	2,500	870	1,400	320	0.57	0.11	2.78	1.10	910	4	
O'Reilly <i>et al.</i> ²²	1,100									14	
Satellite data	1,000	620	280	190	0.27	0.07	1.05	0.58	980	25,974	Stratified
1985 cruise data	1,300	260	490	180	0.41	0.20	1.12	0.22	1,100	3	
O'Reilly <i>et al.</i> ²²	870	340								11	

The f -ratios cited from the 1985 cruise are based on tracer ^{15}N uptake measurements. Note that, when the production estimates are divided by the biomass, the estimates from compound remote sensing and the *in situ* observations become more similar to each other, which suggests that biomass variability is a principal source of variation in primary production at the scales considered here. Note also the extreme undersampling of the ship observations compared with the satellite ones. The results calculated from O'Reilly *et al.*²² are averages for summer months (July–September) for data collected during the period 1977–1982.

* P_t is the total primary production in units of $\text{mg C m}^{-2} \text{d}^{-1}$.

† P_n is the new production in units of $\text{mg C m}^{-2} \text{d}^{-1}$.

‡ $f\text{-ratio} = P_n/P_t$. Dimensionless.

§ B is the pigment concentration in mg m^{-3} .

|| X is the ratio of mean P_t to mean B in units of $\text{mg C m}^{-2} \text{d}^{-1} (\text{mg Chl m}^{-3})^{-1}$.

¶ n is the number of observations: for satellite data, it is the number of pixels.

ocean colour can be used³ to estimate P_t , allowing P_n to be found as the product of f and P_t .

The principal data base of *in situ* observations for this study consists of material collected during two cruises of CSS Hudson (23 July–12 August 1985 and 16–30 August 1988) on which measurements were made of P_t (^{14}C method) and P_n (^{15}N method). We have also referred to earlier data sets, in particular that of O'Reilly and Busch¹⁴. For ocean colour, no CZCS was active in 1988. Accordingly, we searched the NASA archive¹⁵ for images covering Georges Bank collected on cloud-free days during July and August 1985. Among these, we selected for analysis those images corresponding to days for which we had access also to the relevant AVHRR data. We present here the results of calculations carried out for 7 August 1985, which had the best cloud-free images.

Application

In summer, the mixed waters on Georges Bank are separated from the surrounding stratified waters by an annular frontal zone, whose properties are intermediate between those of the well mixed and stratified water types. The three zones are seen clearly in the satellite-derived biomass (B) and SST fields for the region on 7 August 1985 (Fig. 1). The central, mixed waters are characterized by high B and high T , the annular front by lower B and lower T (because of frontal upwelling) and the stratified waters by high T and relatively low B . Satellites supply data on T and B at the surface only, whereas the primary production extends over a finite depth of the water column (the euphotic zone), and we therefore need a method of extrapolating the surface values over the entire range of interest of depth z . This problem is trivial for the mixed waters, which show little vertical structure in either T or B . The mean temperature and biomass profiles for the stratified waters, as well as for the adjoining Sargasso Sea and Slope waters, based on shipboard measurements, are shown in Fig. 2. Clearly, these zones are distinct from each other not just in surface characteristics, but in vertical structure as well. The vertical structure in the frontal region (not shown) is complex, oscillating between that of the mixed waters and that of the stratified waters, depending on the state of the tide.

On each cruise, many nitrate measurements were made on water taken from a CTD (conductivity–temperature–depth) rosette sampler. The samples were analysed at sea by industrial method 158-71W using an autoanalyser. In each year, it was found that T was an excellent predictor for nitrate concentration (Fig. 3a). Pastuszak *et al.*¹⁶ have shown that this temperature dependence varies with season, an observation supported by

our own measurements (understandably, the deep, cold waters show little seasonal dependence, but the surface, nitrogen-depleted waters become warmer as the summer progresses). We have therefore used the relationship between T and N for 1988, which is based on August data, rather than the 1985 field data, which were collected in July. In other words, we have made the assumption (borne out by observations from cruises) that the critical algorithms, established during July and August in one year, can be applied to satellite data from a different year. Our field data also show (Fig. 3b) the dependence of f on N as found elsewhere¹².

The protocol we used to estimate P_n combines these *in situ* observations with the satellite data. The satellite-derived SST and the local bathymetry field delineate the mixed waters ($T > 16^\circ\text{C}$, $z < 45\text{ m}$), stratified waters ($T > 16^\circ\text{C}$, $200 < z < 45\text{ m}$), and frontal waters ($T < 16^\circ\text{C}$, $z < 200\text{ m}$), as shown in Fig. 4. The balance of the region was assigned either to Slope waters ($200 < z < 2,000\text{ m}$) or Sargasso Sea waters ($z > 2,000\text{ m}$). For each pixel in the study area, the biomass profile was estimated from the CZCS data and the typical profile shape for the region, using the method of Sathyendranath and Platt¹⁷. The temperature of the mixed layer was estimated from the satellite data, and the temperature profile below the mixed layer was assumed to be the mean temperature profile for the region. For the frontal region, we assumed that the vertical structure had the characteristics of the stratified waters for one half of the tidal cycle, and those of the mixed waters for the other half. Cloud cover for the region in August was estimated from climatological data¹⁸ to be 0.5. The primary production $P_t(z)$ at each depth z was calculated according to the procedure of Platt and Sathyendranath^{3,19}. The rate constants of primary production necessary for these computations are available from measurements made during the 1985 cruise²⁰; they show little dependence on water type. The new production was then calculated as $P_n(z) = f(z) \times P_t(z)$, where $f(z)$ was estimated from the relationships between N and T , and between f and N .

Results and discussion

Depth-integrated values of P_t and P_n are presented in Fig. 5a and b. Their ratio, P_n/P_t , gives the f of Fig. 5c. There is an area of high primary production ($\sim 1,500\text{ mg C m}^{-2} \text{d}^{-1}$) over the bank, surrounded by a ring of lower production ($\sim 1,000\text{ mg C m}^{-2} \text{d}^{-1}$). Further away from Georges Bank, P_t is still lower ($400\text{--}800\text{ mg C m}^{-2} \text{d}^{-1}$). Some similarities with the biomass image are obvious in a comparison with Fig. 1a, but the primary production field is not just a remapping of the biomass field in different units²¹. As cloud cover is assumed to

be uniform over the region, the incoming solar radiation is also uniform, and therefore the differences between the P_t and B fields are mostly due to differences between water masses in the vertical structure of the B field.

Figure 5b shows clearly that the mixed and frontal waters of Georges Bank are important zones of new production, in comparison with the surrounding stratified waters. The interesting point is that the maxima in P_n do not necessarily coincide with the maxima in P_t : the region of maximum P_t (in shades of red in Fig. 5a) has relatively low values of P_n .

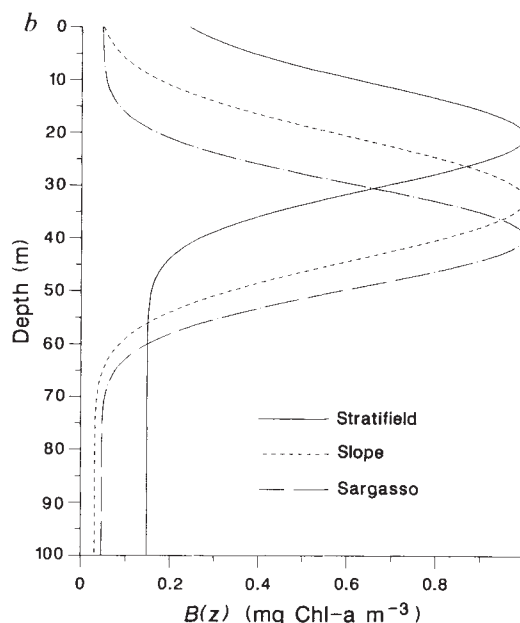
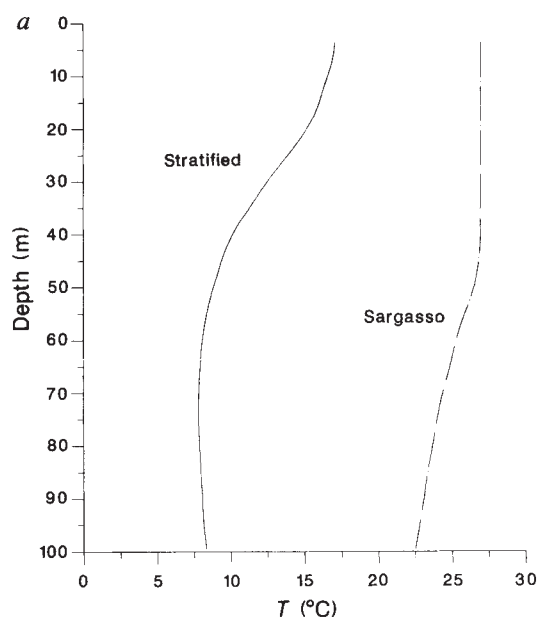


FIG. 2 Mean vertical structure of the water column, based on *in situ* measurements. Mixed waters are not shown, as they are assumed to have no vertical structure. *a*, Temperature structure for stratified and Sargasso Sea waters. For Slope waters we have assigned the same mean characteristics as for

the Sargasso Sea waters. *b*, The typical biomass profiles for Slope, Sargasso Sea and stratified waters. All three profiles are normalized to unity at the maximum, to emphasize the variations in the shape rather than in the magnitude of the chlorophyll values.

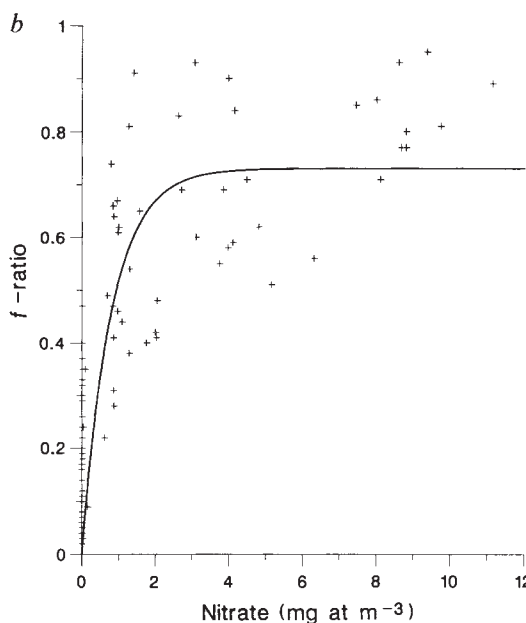
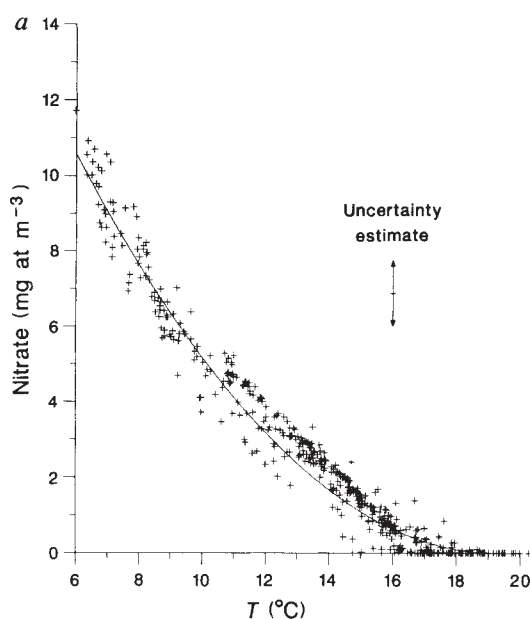


FIG. 3 Relations between temperature, nitrate and f -ratio on Georges Bank. *a*, T against N , based on *in situ* data collected during August 1988. Data from all depths are included. The fitted equation (continuous line)¹¹ is $N = 21.1(\pm 0.28) - 2.08(\pm 0.04)T + 0.0505(\pm 0.0017)T^2$, where T is in $^{\circ}\text{C}$

and N is in mg-at m^{-3} . *b*, f against N from data collected during the 1985 cruise. The fitted equation (continuous line)¹² is given by $f = 0.731(\pm 0.036)[1 - \exp(-1.25(\pm 0.237)N)]$.

capacity of the system to support production at higher trophic levels. It is noteworthy, then, that the northeastern portion of the Georges Bank, with a pronounced maximum in f , is also the region of highest local abundance of the scallop (*Placopecten magellanicus*), an important exploited species¹¹.

Our estimates of average productivity of the Georges Bank waters, and earlier estimates based on *in situ* data for the region, are shown in Table 1. Comparing these results is difficult, not least because the satellite data are based on many pixels scanned on a single day, whereas the *in situ* estimates are based on a handful of stations occupied over a period of days, if not months. The question arises, however, as to which results are more representative of the regional primary production. In our opinion²¹, the estimates that have made use of remote sensing should be considered the more reliable. Our approach makes use of all the relevant *in situ* measurements that are available;

remote sensing serves to extend the data in the horizontal dimension, and thus to account for small- to meso-scale variability in biomass. In other words, remote sensing should be at least as good as any method based on ship observations alone, and will be the more advantageous as the biomass field becomes more heterogeneous and undersampling becomes more acute for ship operations. For calculation of primary production on a single day, and for a small region like the Georges Bank, light available at the surface can be assumed spatially constant. Of the other variables responsible for changes in ocean productivity at these scales, biomass seems to be the most important^{21,23-25}. Oceanic biomass varies over an extremely wide range of spatial and temporal scales, and not all of these have been accessible to direct measurement. Remote sensing allows us to fill in the gaps¹⁵. To account for spatial variability at the kilometre scale and temporal variability on scales of days and months, remote

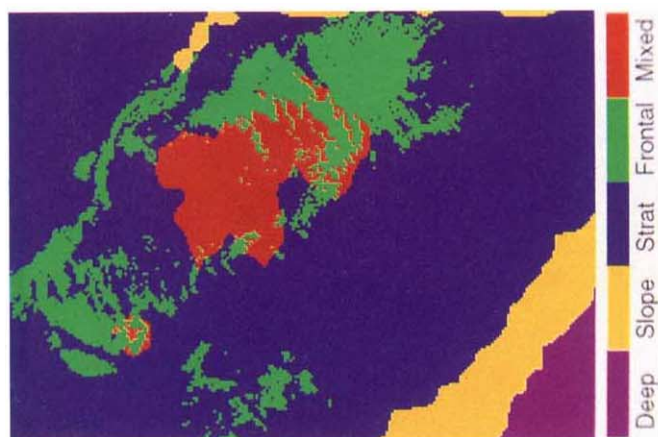


FIG. 4 Water types near Georges Bank. Frontal waters, mixed waters, stratified waters, Slope waters and Sargasso (deep) waters are identified by different colours. The area covered by the image corresponds to the boxed region in Fig. 1a. The image shows a ring of frontal waters separating the mixed and stratified waters.

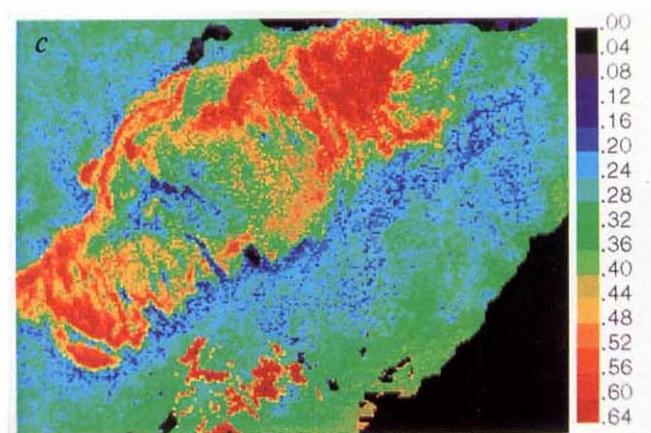
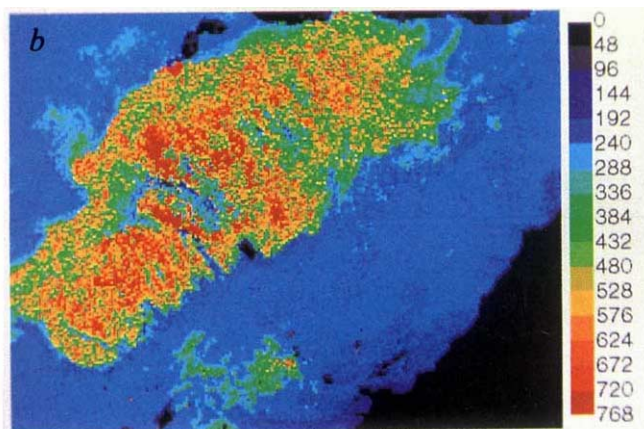
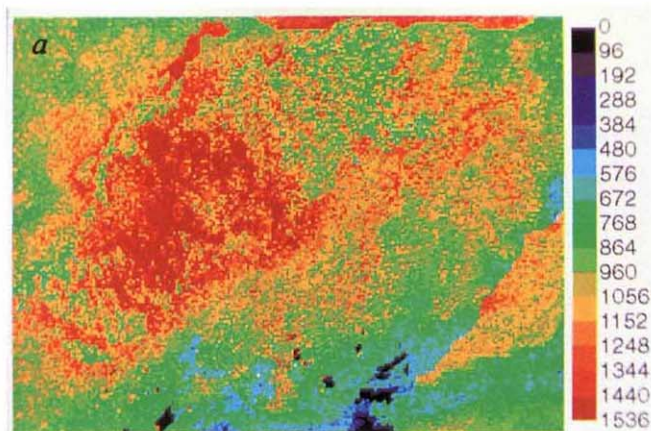


FIG. 5 Computed primary production on the Georges Bank for 7 August 1985. The region covered corresponds to the box in Fig. 1a. a, P_t in daily rates integrated over the water column ($\text{mg C m}^{-2} \text{d}^{-1}$). b, P_n , also in $\text{mg C m}^{-2} \text{d}^{-1}$. The figure shows P_n for the Slope and Sargasso Sea waters (extreme south eastern part of image) to be virtually zero. This is likely to be an error, however, as the T against N relationship used in this work is based only on data from the Georges Bank region. The Slope and Sargasso Sea waters, with their higher temperatures (Fig. 1b), would require a different empirical relationship to link T and N . c, The f -ratio. Note that $P_n = f \times P_t$.

sensing is the only available method. When using *in situ* data, which are necessarily limited in number, one is often compelled to combine data from many years to obtain a coherent picture of a region. The results are at best a multi-year average, hardly suitable for studies of climate change: in this context too, remote sensing seems to be the only solution.

To assign probable errors to the calculated values of P_n , we first calculate the variance associated with the estimation of N from T (Fig. 3a) as $\text{var}(N) \sim (\partial N / \partial T)^2 \text{var}(T) + \sum_{i=1}^3 (\partial N / \partial x_i)^2 \text{var}(x_i)$ where the x_i are the coefficients of the second-degree polynomial used to fit the data. If we assume²⁶ that the satellite-derived temperature field is good to within 0.5 °C (that is, $\text{var}(T) = 0.25$), we find that for $12 < T < 18$ °C, the probable error in N varies over the narrow range from 0.7 to 0.9 mg-at N m⁻³. Using these error estimates, and following the same procedure with Fig. 3b, we find that the probable error in f is only 0.03 for $N > 2$ mg-at m⁻³, but increases for lower nitrate concentrations, reaching 0.3 at $N = 0.7$ mg-at N m⁻³. We can now calculate the probable relative error in new production for an individual pixel, $\varepsilon(P_n)$, as $\sqrt{(\varepsilon(f))^2 + (\varepsilon(P_t))^2}$. Using a value²⁷ of $\varepsilon(P_t) = 0.42$, we find $\varepsilon(P_n)$ to be 43% at $N = 2$ mg-at N m⁻³ and 85% at $N = 0.7$ mg-at N m⁻³. In other words, the error in P_n is dominated by the error in P_t at high N , and by the error in f at low N . The two terms contribute equally to the error at $N = 1.0$ mg-at N m⁻³. These conclusions, qualitative and quantitative, would not change materially if the precision of the SST measurements were improved, say, by a factor of two: it would confer no benefit at high N , and at low N the errors associated with the parameters of the f - N relation matter more than those associated with N itself (a variable derived from SST).

We should compare these figures with the probable relative error implicit in a calculation of P_n by ship measurements alone. Lack of knowledge of the spatial variances in f and P_t would preclude its estimation in a typical field application, but we can proceed on the basis of the information in Table 1. Ignoring the experimental errors, we consider only those errors arising from the local variations in f and P_t . We find that spatial heterogeneity leads to relative errors in P_n of 61%, 48% and 67% for the mixed, frontal and stratified regions, respectively, and that covariance between f and P_t makes negligible contribution to $\varepsilon(P_n)$. These errors are higher than that associated with a single pixel in the compound remote sensing method when N

is high, but do not increase as N decreases.

Hence, the estimation error of the compound remote sensing method, locally applied, is roughly commensurate with the (usually unknown) spatial error of the conventional ship method, regionally applied. To find a regional average with satellite data, the remote-sensing method is applied to a large number of pixels n . Granted that the n points do not constitute a random sample, and that the probable error may not therefore decrease as fast as $1/\sqrt{n}$, it seems intuitively clear to us that the n estimates will lead to a more reliable regional average (with quantifiable variance) than that (with unknown variance) obtainable from a few ship estimates.

Our method of using compound remote sensing to estimate new production is based on simple empirical relationships, and does not require a suite of assumptions regarding the kinetics of nitrate uptake, as in the method of Dugdale *et al.*⁷. Relationships between N and f have been reported for other regions^{13,28-30} and temperature-nitrate relationships have also been observed for various oceanic localities^{31,32}. The general method presented here should therefore be applicable to other regions. It might be argued that our approach breaks down in those regions where f does not appear to depend on N (refs 33, 34) and in latitudes where nutrients may not limit production³⁵. In such cases, the solution would be to look for other proxy variables that are accessible to remote sensing, and it seems that alternatives do exist: for example, Le Bouteiller³³ has suggested that chlorophyll concentration itself would be the best indicator of new production in the equatorial Atlantic ocean. Eppley and Peterson⁹ have shown, on the other hand, that a nonlinear relationship exists between f and P_t , an observation that has been confirmed for other regions¹². As the relationships we use may vary with region and season, it is important that quantitative relationships be established specifically for the region of application. Platt and Sathyendranath³ have suggested that the oceans be divided into dynamic biogeochemical provinces that would be characterized by a given vertical structure in pigment profile and by constant physiological rate constants for primary production. This work suggests that, if the properties of these provinces are extended to include vertical temperature structure and temperature-nitrate- f relationships, it should be possible to calculate new production at large horizontal scales from remote sensing. This would be a considerable step towards estimation of global new production. □

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MHC class II structure, occupancy and surface expression determined by post-endoplasmic reticulum antigen binding

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Class II major histocompatibility complex molecules undergo a change in structure upon stable binding of peptide antigen. Analysis of the site and extent of this change among class II molecules of splenic antigen-presenting cells reveals the preference of class II for peptide acquisition outside the endoplasmic reticulum and indicates that the class II presentation system is not saturated with self peptides. There are numerous empty class II molecules on the cell surface and peptide antigen is evidently important in regulating surface class II expression.

CLASS I and class II major histocompatibility complex (MHC) molecules have a similar function, which involves the display of antigenic peptides for recognition by T cells¹⁻⁵. Experiments with cell lines defective in class I molecule assembly have demonstrated the critical role of peptide in stabilizing class I heavy chain- β_2 -microglobulin complexes and in facilitating their movement to the surface⁶⁻¹⁰. By analogy with cells defective in presenting endogenous antigen in association with class I and possessing unstable class I molecules, it has been shown that cells deficient in presenting exogenous protein antigens have class II molecules that are less stable than those of normal cells¹¹. Nevertheless, antigen-dependent intracellular changes in class II analogous to those described for class I have not been reported, so that the extent and the site(s) of peptide-driven effects on class II structure and transport remain unknown. Such studies are made particularly important by reports of effective class II presentation of peptides derived from intracellular proteins¹²⁻¹⁸, which suggest that class II molecules may form long-lived complexes with peptide in the endoplasmic reticulum (ER) as well as in post-Golgi acidic compartments.

Using purified class II molecules, we have obtained evidence^{6,5} that the stable binding of peptide is necessary for class II $\alpha\beta$ dimers to enter a compact state that resists dissociation in 1-2% SDS at room temperature (C state)¹⁹⁻²³. Here we use this relationship between peptide binding and class II structure to investigate at what point after initial biosynthesis, to what extent, and with what consequences class II molecules stably associate with processed antigen. On the basis of this criterion, we find no evidence for substantial tight peptide binding to class II molecules before transit through the medial Golgi. Conversion of class II dimers to the compact, stable state is a chloroquine-sensitive process that only occurs with molecules free of intact invariant chain (Ii) and only involves a fraction of newly synthesized class II molecules. Addition of antigen augments the generation of stable class II dimers and increases overall surface class II molecule expression. Many class II molecules failing to capture peptide are degraded, but a substantial number of apparently empty class II molecules reach and reside on the cell surface.

Newly assembled class II dimers are unstable

Spleen cells were pulse-labelled and class II proteins immunoprecipitated from lysates prepared immediately after labelling or after a 4-hour chase. Figure 1a gives representative results for $A\alpha^kA\beta^k$, $A\alpha^dA\beta^d$, $E\alpha^kE\beta^k$ and $E\alpha^dE\beta^d$. No class II dimers stable in SDS at room temperature are seen immediately after pulse labelling, although all precipitates show α , β , and Ii proteins (U dimers). Because chain-specific monoclonal antibodies were used, the coprecipitation indicates that associated $\alpha\beta$ complexes are present at this time²⁴, but they have not attained a denaturant-resistant form. In contrast, after the chase, the class II molecules show significant amounts of C dimers migrating with an apparent relative molecular mass (M_r) of 52,000-56,000 (52K-56K). The ratio of C to U complexes varies with the class II allele and isotype, but is similar for the same $\alpha\beta$ combination from cells of distinct mouse strains (for example, compare $A\alpha^kA\beta^k$ and $E\alpha^kE\beta^k$ for C3H, CBA and B10.BR in Fig. 1 a, b and c, respectively).

Binding-site conformation distinguishes dimers

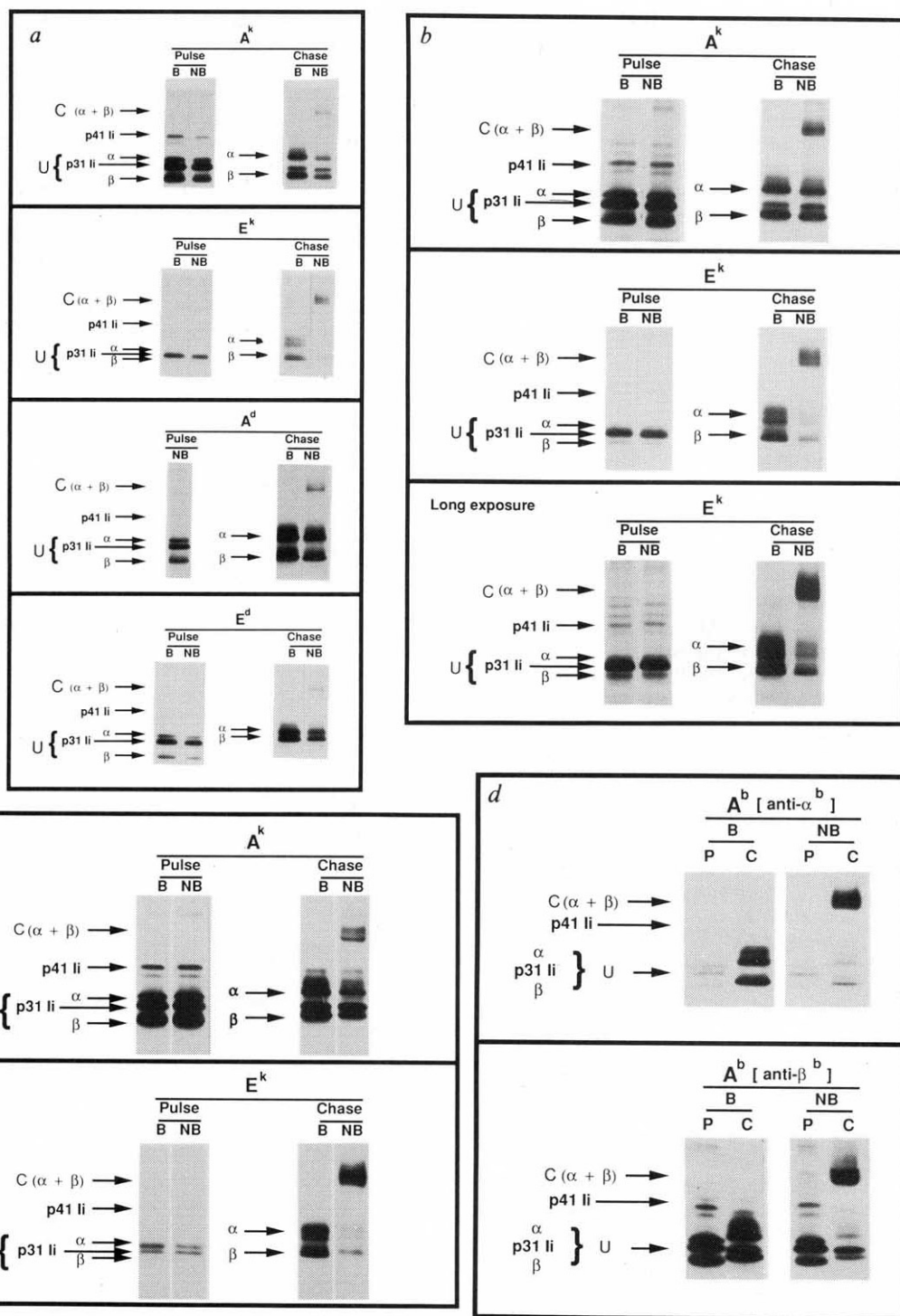
There was little precipitation of $E\alpha E\beta$ after pulse-labelling, whereas chased samples gave strong signals (see Fig. 1a, b and c for E^k). This is not a technical artefact, because total counts precipitable with trichloroacetic acid do not increase between the pulse and chase and the $A\alpha A\beta$ precipitates from the same lysates do not show this effect (Fig. 1a-c). The explanation became clear after analysis of $A\alpha^bA\beta^b$ molecules using monoclonal antibodies with differing sensitivities to the structure of the peptide-binding region. Y3P (ref. 25) is an anti- $A\alpha^b$ reagent whose binding depends on the conformation of the assembled $\alpha\beta$ complex²⁶. M5/114 (ref. 27) is an $A\beta^b$ -specific antibody whose binding depends on local β -chain structure, reacting with numerous $\alpha\beta$ combinations^{26,27} and with free $A\beta$ chain (unpublished observations). Immunoprecipitation with the anti- $A\alpha^b$ antibody demonstrates an increase in class II between the pulse (P) and chase (C) samples (Fig. 1d, top). Virtually all the increase is due to the appearance of C dimers during the chase. In contrast, with the anti- $A\beta$ antibody there is a similar intensity of bands in the pulse and the chase samples, which resemble the chased sample intensity in the anti- $A\alpha$ precipitated lanes. These data indicate that the ability of some antibodies to bind class II is altered by a conformational difference between newly assembled dimers and these same dimers several hours later. Further, the gain in serological reactivity is almost exclusively associated with the acquisition of the C state that corresponds to long-lived peptide-class II complexes. Because the 14-4-4S and Y3P antibodies are more reactive with C dimers, this species is emphasized in immunoprecipitations performed with these reagents. Yet in the precipitates from pulse-labelled cells synthesizing $A\alpha^bA\beta^b$, $E\alpha^kE\beta^k$ or $E\alpha^dE\beta^d$, only chains from U dimers are seen. Thus, despite a bias towards the C form, such molecules are undetectable among newly assembled $\alpha\beta$ dimers, as demonstrated in Fig. 1b (long exposure).

FIG. 1 Post-assembly changes in MHC class II dimer structure and antibody reactivity. Metabolically labelled class II molecules and associated proteins were immunoprecipitated from lysates of pulse-labelled or pulse-labelled and chased spleen cells derived from *a*, C3H (H-2^k) or DBA/2 (H-2^d) mice; *b*, CBA (H-2^k) mice; *c*, B10.BR (H-2^k) mice; or *d*, C57Bl/6 (H-2^b) mice. Immunoprecipitations with the anti-A^B^k antibody 10-2.16 (refs 26, 60) are labelled A^k; those with the anti-A^B^d antibody MKD6 (refs 26, 61) are labelled A^d; those with the anti-E^a antibody 14-4-4S (ref. 62) are labelled E^k and E^d; those with the anti-A^a^b antibody Y3P (refs 25, 26) are labelled A^b [anti- α^b]; and those with the anti-A^b^b antibody M5/114 (refs 26, 27) are labelled A^b [anti- β^b]. C ($\alpha + \beta$) identifies C-state $\alpha\beta$ dimers whose structure depends on tight peptide binding⁶⁵; p41 li and p31 li refer to the products of the two alternatively spliced mRNAs of the mouse invariant chain gene³⁴; U refers to the α and β chains derived from U state dimers that dissociate in SDS and correspond to molecules lacking tightly associated peptide⁶⁵. Lanes labelled B correspond to samples that have been heated to 100 °C before SDS-PAGE; lanes labelled NB correspond to samples kept at room temperature before SDS-PAGE.

METHODS. Cell from spleens of normal mice of the C3H, CBA, DBA/2, B10.BR or C57Bl/6 strains were washed at 37 °C in PBS with 2% fetal calf serum, then resuspended in pre-warmed leucine-deficient RPMI-1640 medium containing 5% dialysed fetal calf serum at 2×10^7 per ml. After incubation at 37 °C for 1–2 h, cells were pelleted at room temperature for 10 min then resuspended in leucine-deficient RPMI-1640 with 5% dialysed fetal calf serum containing 200–300 μ Ci of ³H-leucine per ml. Cells were incubated for 20 min at 37 °C then divided into aliquots for further treatment. One aliquot was collected rapidly by centrifugation in cold medium and the cell pellet immediately

lysed in 1% NP-40, 0.006M CHAPS, 50 mM Tris, pH 8, 0.15 M NaCl, 5 mM EDTA, 50 μ M PMSF, 50 μ g ml⁻¹ TLCK (N- α -p-tosyl-L-lysine chloromethyl ketone) and 1:500 aprotinin (Sigma) (cell lysis buffer) at a concentration of 5×10^7 cells per ml ('Pulse'); the other aliquot of cells was spun at room temperature and resuspended at the original cell concentration in pre-warmed RPMI-1640 medium with 10% fetal calf serum and 10 $\times$ cold leucine. After incubation at 37 °C for a further 4 h, cells were collected by centrifugation and lysed as before ('Chase'). Cell lysates were cleared of nuclei and debris by centrifugation, then pre-treated with normal rabbit serum, rabbit anti-mouse immunoglobulin and PanSorbin (CalBiochem) overnight at 4 °C. After one additional incubation with PanSorbin and one with protein A-Sepharose, the lysate was used for immunoprecipitation without freezing. This was accomplished either by (1) addition of the monoclonal antibodies to a volume of lysate representing 1–2 $\times 10^7$ cells per lane on the final SDS-PAGE, incubation at 4 °C overnight, then addition of 100 μ l of a 50% slurry of protein A-Sepharose per 1–2 $\times 10^7$ cell equivalents, and further

incubation at 4 °C for 1 h, or (2) addition of 100 μ l of a 50% slurry of protein A-Sepharose to which antibody had been previously bound per 1–2 $\times 10^7$ cell equivalents, followed by incubation at 4 °C for 2 h to overnight at 4 °C. In the case of the rat antibody M5/114, rabbit anti-rat IgG was prebound to the protein A-Sepharose beads. Immunoprecipitates bound to protein A-Sepharose were washed in 0.006M CHAPS, 50 mM Tris, pH 8, 0.15 M NaCl and 5 mM EDTA, and the washed beads resuspended in 100 μ l sample buffer containing 2% SDS, 62.5 mM Tris, pH 6.8, 10% glycerol and 0.77 M 2-mercaptoethanol. One half of the sample was heated to 100 °C for 2 min (B); the other left at room temperature for 1 h (NB). The eluted proteins were then analysed by SDS-PAGE in 10% gels as described⁶³. Gels were treated with Enhance (DuPont-NEN), dried, and autoradiographs prepared. All lanes for each antibody and cell combination shown come from a single autoradiographic exposure. The immunoprecipitates and SDS-PAGE are the same in the bottom two sections of *b*, labelled E^k, but the autoradiographic exposure was 3 $\times$ as long for the bottom section (labelled 'long exposure').



Mutually exclusive C state and li-association

Newly synthesized class II α and β chains associate with li in the ER^{24,28-30}. Because we did not observe any C dimers early after such ER assembly, and because recent *in vitro* studies^{31,32} suggest li can inhibit the tight peptide-class II molecule association that this state represents, we examined whether li-associated class II could achieve the C conformation *in vivo*. $E\alpha^kE\beta^k$ -expressing spleen cells were pulse labelled, lysed, or chased before lysis, and proteins immunoprecipitated with 14-4-4S to provide maximum detection of C forms. No C dimers are seen in the pulse-labelled sample (Fig. 2). As early as 30 min of chase (lane C.5), a significant amount of these dimers is present, which increases over the 4 hours of chase. Precipitation with the P4H5 monoclonal antibody against mouse li³³ reveals large amounts of p31 and p41 li (ref. 34), as well as li disulphide dimers, all of whose levels decrease with time of chase. Despite significant amounts of li-associated class II molecules of mature glycosylation type at 1 h of chase (C1), no C dimers are seen in these complexes and this is true even at 4 h. Conversely, after preclearing of all immunoreactive li and associated class II (last panel), precipitation of the remaining $E\alpha E\beta$ proteins with 14-4-4S shows the same level of C dimers at each time as is seen without removing the li-associated class II molecules. Thus, even at late times after initial assembly and even after transport out of the ER, $\alpha\beta$ dimers associated with li do not convert to the C state, and all detectable C dimers are free of intact li.

Small amounts of $E\alpha$ and $E\beta$ of lower apparent M_r indicative of a pre-medial-Golgi location are present in the anti-li precleared lysates from pulse-labelled cells immunoprecipitated with 14-4-4S (E^k (anti-li pre-clear), P). li is absent from this lane, confirming the completeness of the preclearing steps. Such chains might derive from dimers dissociating from li during the preclearing steps. In this case, the absence of C dimers would be expected. Alternatively, these may represent $\alpha\beta$ dimers that either have not yet associated with li or that are destined to exit the ER without li. The absence of C forms would then imply that class II, even without li inhibition of peptide association, does not attain in the ER the structure associated with tight peptide binding.

Exogenous antigen and generation of C dimers

Not all class II achieves the C state during 4 hours of chase, even after li dissociation. One possible explanation is that there is insufficient peptide available to occupy all newly synthesized class II. This was examined by incubating spleen cells from

mouse strain CBA in 2 mg ml^{-1} of hen egg lysozyme (HEL), which is efficiently taken up and processed into peptides that strongly bind to $A\alpha^kA\beta^k$ (refs 3, 35, 36). The addition of HEL leads to an 8-fold increase in immunoprecipitable $A\alpha^kA\beta^k$, and virtually all of the increase is in the C form (Fig. 3a). To test whether this was due to nonspecific induction of class II biosynthesis and/or a change in cell physiology that favoured conversion of class II to the C state, we carried out pulse-chase experiments using both HEL and proteins (pigeon cytochrome c in this case) that have not been found to prime $A\alpha^kA\beta^k$ -restricted T cells or to produce peptides binding to the $A\alpha^kA\beta^k$ molecule³⁶. Culture of CBA spleen cells in HEL, but not pigeon cytochrome c, significantly augments the appearance of C dimers beginning at 1 h of chase, with little change in class II in the pulse-labelled sample (Fig. 3b). Repeat experiments demonstrate that the small increase in $A\alpha^kA\beta^k$ precipitated from the lysate of pulse-labelled cells is not consistent, despite the reproducible and large increases (5–10 fold) in C forms (for example, see Fig. 4). Furthermore, HEL fails to increase $A\alpha^dA\beta^d$ C forms in H-2^d or H-2^{d×k} cells, even though substantial increases in $A\alpha^kA\beta^k$ C forms are seen in the latter (data not shown). These results make it unlikely that nonspecific class II upregulation is mediated by the HEL itself or by contaminants such as lipopolysaccharide.

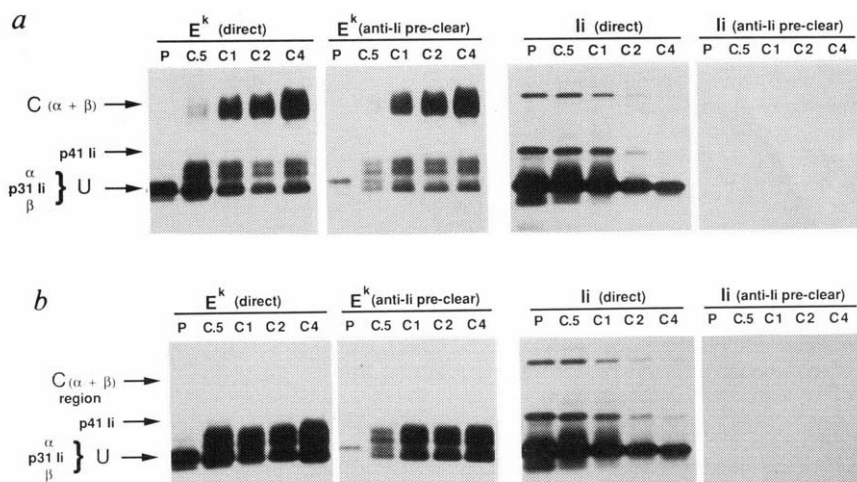
Figure 3b also makes two other important points. First, in the absence of HEL, total precipitable $A\alpha^kA\beta^k$ decreases with time, indicating degradation or shedding. Conversion of immunoprecipitable U molecules to the C state accounts for only a fraction (20–30%) of the HEL-induced C dimers. Thus, HEL appears to rescue class II destined for rapid degradation and converts it to a form that is both biochemically and biologically stable. Second, by 2 h of chase, all class II chains show a migration pattern indicative of passage through the medial Golgi. All the C dimers migrate with the same R_f , irrespective of when they appear (compare 1 h of chase with 18 h). This is evidence that all C dimers are formed from mature glycosylated class II that has passed through the medial Golgi, which is consistent with evidence that li dissociation also occurs after class II transits the Golgi and intersects endocytic or lysosomal compartments^{37,38}.

Chloroquine-sensitive generation of C dimers

Chloroquine is a prototypic inhibitor of antigen processing and presentation by class II (ref. 3) and should inhibit the HEL-mediated increase in C forms of $A\alpha^kA\beta^k$ if this is the result of

FIG. 2 Compact SDS-stable dimers are found exclusively among class II molecules lacking associated invariant chain. CBA spleen cells were metabolically pulse-labelled (P) or pulse-labelled and chased for 0.5 h (C.5), 1 h (C1), 2 h (C2) or 4 h (C4). $E\alpha^kE\beta^k$ molecules and associated proteins were immunoprecipitated with 14-4-4S either directly from untreated lysates of labelled cells (E^k (direct)), or from lysates cleared of li and associated class II molecules using an anti-li antibody (E^k (anti-li pre-clear)). Labelled proteins immunoprecipitated by the first anti-li antibody treatment (li (direct)) and the last anti-li antibody treatment (li (anti-li pre-clear)) are also shown. **a**, Results with samples eluted at room temperature; **b**, results of samples heated at 100°C before analysis. The labelling of the bands is the same as in Fig. 1.

METHODS. Labelling, immunoprecipitation, SDS-PAGE and autoradiography were performed as described in the legend to Fig. 1, except that (1) after 20 min of pulse-labelling, the chase period was 0.5, 1, 2 or 4 h, and (2) invariant chain and associated class II proteins were immunoprecipitated directly from untreated lysates using the hamster anti-li antibody P4H5



(ref. 33) or removed from the lysates before 14-4-4S precipitation by four sequential immunoprecipitations using P4H5.

peptide–class II association. Figure 4 shows that the HEL-driven increase in C forms is eliminated by this drug without a corresponding decrease in class II biosynthesis. In untreated cells, stable dimers appear only after the chase and only among Ii-unassociated $\alpha\beta$ complexes (compare P and C lanes for the upper, middle and lower panels). Culture in chloroquine for 1 h before and during pulse labelling results in a slight reduction in class II biosynthesis (P lane), and continued culture in chloroquine during the 4 h chase causes almost complete loss of the stable form (C lane). This ability of chloroquine to inhibit constitutive formation of C dimers is consistent with other reported data¹¹. HEL increases the pool of C molecules markedly without any increase in the initial biosynthetic rate (compare lanes P and C for untreated and HEL in the upper panel), and this occurs in the Ii-unassociated cohort (middle and bottom panels). The presence of chloroquine along with HEL (H+C lanes) again leads to an absence of C dimers and a prolongation of Ii-class II association³⁸ (bottom panel).

Increases of surface MHC class II expression

Our results show that antigen (HEL) increases the pool of biochemically stable $A\alpha^kA\beta^k$ and that these dimers also have a longer biological half-life than the class II from which they derive. To explore whether these additional molecules contribute to class II surface expression, CBA cells were incubated overnight in complete medium with or without HEL and stained for $A\alpha^kA\beta^k$ (Fig. 5). Incubation in HEL increases surface $A\alpha^kA\beta^k$ expression to between 140–220% of the level on cells cultured without additional antigen. Proteins such as pigeon cytochrome c and ovalbumin, which do not increase the number of biochemically stable $A\alpha^kA\beta^k$ molecules, also do not increase surface $A\alpha^kA\beta^k$ expression, and only $A\alpha^kA\beta^k$ but not $A\alpha^dA\beta^d$ expression is increased on H-2^d_x spleen cells by HEL (data not shown).

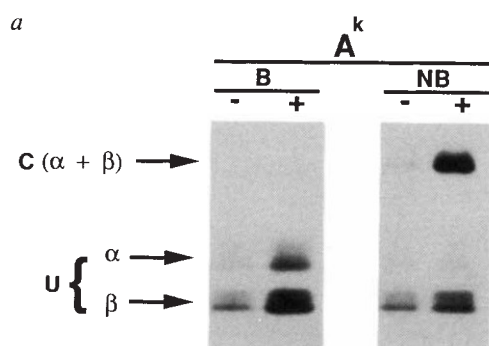


FIG. 3 Exogenous antigen increases generation of compact stable class II dimers. *a*, CBA spleen cells were pre-incubated either with (+) or without (–) hen egg lysozyme for 3 h, pulse-labelled with ³H-leucine, and chased overnight in the continued presence or absence of HEL. Labelled $A\alpha^kA\beta^k$ and associated proteins were immunoprecipitated from the lysates of these cells with anti- $A\beta^k$ antibody and analysed by SDS–PAGE either after elution with heating to 100 °C (B) or elution at room temperature (NB). *b*, CBA spleen cells were pre-incubated for 3 h either in complete medium without added antigen (None), with added hen egg lysozyme (HEL), or with added pigeon cytochrome c (PCC). They were then pulse-labelled or pulse-labelled and chased for 1 h (C1), 2 h (C2), 4 h (C4), 8 h (C8) or 18 h (C18), in the presence or absence of antigen, lysed, and $A\alpha^kA\beta^k$ molecules and associated proteins immunoprecipitated using the anti- $A\beta^k$ antibody 10-2.16. Labelled proteins in immunoprecipitates eluted at room temperature were analysed by SDS–PAGE. Labels on the left are as in Fig. 1. Labels on the right indicate the positions of $A\alpha^k(\alpha_m)$ and $A\beta^k(\beta_m)$ chains migrating with the apparent M_r of proteins that have undergone complex carbohydrate addition in the medial Golgi.

METHODS. For the experiment shown in *a*, CBA spleen cells were pre-cultured for 3 h at 37 °C in the presence or absence of 2 mg ml^{–1} hen egg lysozyme, pulse-labelled with ³H-leucine in the presence or absence of antigen, then

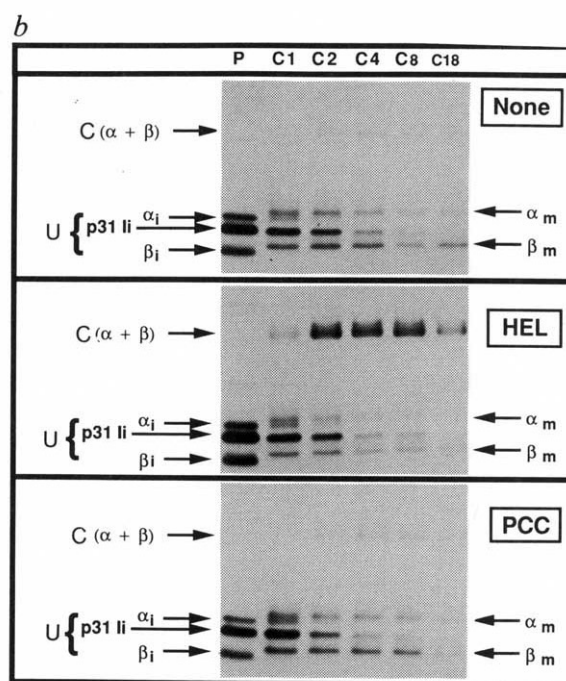
Unstable cell-surface class II molecules

Although these staining experiments, together with the labelling studies, are consistent with antigen-induced C molecules reaching the cell surface, they do not address whether or not all surface class II molecules are of this peptide-associated type. Therefore, surface proteins were labelled on viable H-2^k spleen cells using ¹²⁵I, and the labelled class II precipitated using 10-2.16 or 14-4-4S (Fig. 6). The per cent of C surface dimers resembles that seen at late times (4 h or longer) of chase analysing biosynthetically labelled molecules: about 25–30% C forms for $A\alpha^kA\beta^k$ and 80–90% for $E\alpha^kE\beta^k$. The latter amount is an overestimate of the C fraction because of the bias introduced by use of the 14-4-4S reagent, emphasizing that in both cases a significant proportion of the surface molecules are in the U state associated with the absence of tightly bound peptide.

Discussion

Recent data⁶⁵ establishing a critical role for peptide in producing class II MHC molecules with a characteristic structure provided a means of assessing *in situ* certain features of the class II antigen processing and presentation pathways. Our results using this approach give a number of insights into the post-translation maturation of class II dimers and the quantitative relationships among class II synthetic rates, peptide loading and surface display. They also provide data relating to the stringency with which class II molecules might be prohibited from acquiring peptides in the class I loading compartment (ER)^{1,2,5,12–18}.

It was unexpected that antigen (HEL) would enhance generation of $A\alpha^kA\beta^k$ dimers by 5–10 fold. More modest, less reproducible increases have been seen with other peptide–class II combinations (unpublished observations). This may reflect the fact that HEL is very basic, allowing binding to anionic surface glycoproteins and promoting uptake, and also the efficiency of peptides such as HEL 46–61 to bind $A\alpha^kA\beta^k$ molecules in



incubated for an overnight chase period with or without antigen present. Cells were washed and lysed, and the proteins immunoprecipitated with anti- $A\beta^k$ antibody, the immunoprecipitates eluted at room temperature (NB) or with boiling (B), then subjected to SDS–PAGE followed by autoradiography as described in Fig. 1. For the experiment shown in *b*, the procedure was the same, except that chase periods of 1, 2, 4, 8 and 18 h were used, cultures contained either no antigen or 2 mg ml^{–1} HEL, or 2 mg ml^{–1} pigeon cytochrome c, and all samples were eluted at room temperature.

an acidic environment^{35,36,39}. High HEL concentrations also induced a substantial increase in surface class II; the proportion of surface $A\alpha^kA\beta^k$ containing peptides from this antigen is estimated to be between 20–50%. Under similar conditions, specific antigen occupancy of $Ea^dE\beta^d$ has been estimated at 30% (ref. 40). Selective increases have been reported⁴¹ in surface expression of class II that bind a particular antigen following its administration *in vivo* and $Ea^kE\beta^k$ yields are increased from B cell tumours incubated in cytochrome *c* (ref. 42). These findings indicate that class II production is normally in excess of the functionally available steady-state peptide load, and that peptide binding plays a major part in regulating overall class II levels and surface expression. Because class II binding sites are not normally saturated, foreign peptides do not have to displace self-peptides to bind, explaining to some extent why competition from self-proteins^{5,43} does not pose an overwhelming problem for the class II presentation system.

Peptide stabilizes the conformation of the ligand binding ($\alpha 1/\alpha 2$) region of class I (refs 6–10). Our data indicate that peptide binding also induces a conformational change in the analogous class II $\alpha 1/\beta 1$ region, as indicated by the distinct reaction of the 14-4-4S and Y3P antibodies with newly assembled versus post-Golgi molecules. This change is peptide-dependent but not peptide-specific. Our results complement serological analysis of antigen processing mutants¹¹ and can explain previous observations of monoclonal reagents showing distinct reactivities with early and late post-synthetic forms of class II molecules^{44–46}. Selective purification of peptide-containing C dimers by the particular monoclonal antibodies used to prepare class II molecules for binding experiments may account for the low proportion of available sites seen in many such studies^{36,47}.

No C molecules were associated with intact Ii at any time, which is consistent with data demonstrating that class II-Ii complexes do not bind peptide stably *in vitro*^{31,32}. Although

association with Ii correlates with an absence of conversion to the C state, class II complexed with Ii may be more likely to acquire peptides following Ii dissociation, perhaps by having been brought to a peptide-rich, acidic compartment^{48,49}. This could explain why cells lacking Ii have class II with diminished expression of serological epitopes that might be associated with the C state⁵⁰ and why they are more efficient in presenting exogenous peptides⁵⁰, as might be expected of cells expressing more empty class II.

Equating the compact, biochemically stable, and conformational antibody-reactive C state with avid peptide binding is strongly supported by a variety of data (refs 11, 19, 65). It is less clear whether all molecules lacking these characteristics are devoid of associated peptide. As shown for $A\alpha^bA\beta^b$ molecules, there is agreement between the serological change monitored by the Y3P antibody and the acquisition of the SDS-stable state. Thus, despite the probable existence of a range of peptide-class II binding site affinities even in the C state, the antibody and SDS-gel assays seem to be detecting a discrete change in state of the class II dimer itself that accompanies entry into the stable peptide-binding condition for most of all peptide-MHC combinations. If this is the case, then a substantial fraction of surface class II dimers seem to be unoccupied, based on the proportion of U molecules seen after precipitation of ¹²⁵I-labelled membrane proteins. The alternative interpretation is that some U

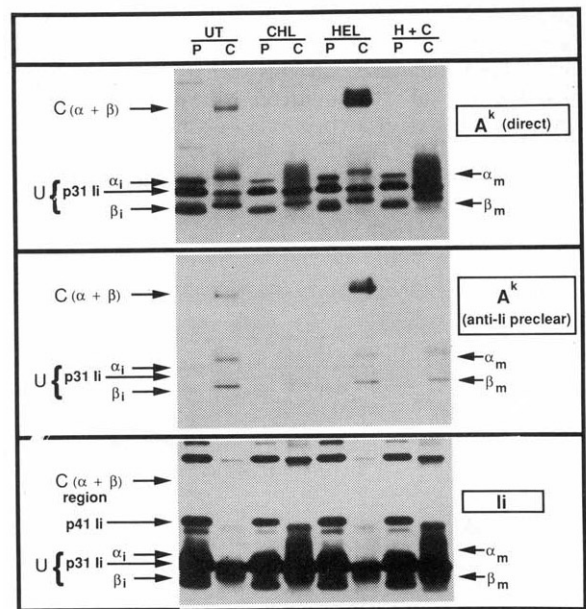


FIG. 4 Generation of compact, stable class II dimers is chloroquine-sensitive. CBA spleen cells were incubated for 1 h in complete medium (UT), in medium with 100 μ M chloroquine (CHL), in medium with hen egg lysozyme (HEL), or medium with HEL and chloroquine (H + C). They were then pulse-labelled under the same incubation conditions, lysed (P) or chased for 4 h in the same culture conditions and lysed (C). $A\alpha^kA\beta^k$ and associated proteins were immunoprecipitated from untreated lysates with 10-2.16 (A^k (direct)) or from lysates from which invariant chain associated proteins had been removed by repeated precipitation with anti-Ii antibody P4H5 (A^k (anti-Ii pre-clear)). These anti- $A\beta^k$ immunoprecipitates and also those from the first anti-Ii pre-clear (li) were eluted at room temperature and analysed by SDS-PAGE. Labels are as in Fig. 1 and 3.

METHODS. All procedures were as described in the legends to Figs 1–3, except that 100 μ M chloroquine was present in some samples during the entire culture period (before, during and after pulse-labelling).

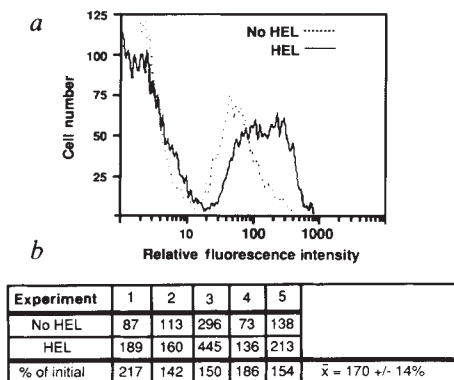


FIG. 5 Antigen increases surface class II expression. *a*, Cell-surface $A\alpha^kA\beta^k$ on CBA spleen cells cultured overnight with or without hen egg lysozyme was measured by flow microfluorimetry using anti- $A\beta^k$ antibody. *b*, The mean fluorescence observed using anti- $A\beta^k$ antibody to stain CBA spleen cells

incubated with or without HEL, and the calculated values of the staining of HEL incubated cells as a per cent of the level on cells cultured without HEL (% of initial), are given for 5 separate experiments.

METHODS. CBA spleen cells were cultured overnight either with or without 2 mg ml⁻¹ HEL. They were washed, incubated with saturating amounts of the anti- $A\beta^k$ antibody 10-2.16, washed, incubated with fluorescein isothiocyanate-conjugated anti-mouse IgG_{2b} antibody, washed, and kept at 4 °C until analysed using a FACScan flow cytometer. Dead cells were gated out using staining with propidium iodide, and red blood cells gated out based on forward scatter. Data on 10,000 gated cells were accumulated for green fluorescence (indicating surface $A\alpha^kA\beta^k$ level) and forward scatter, which provides an indication of cell size. Data are displayed in *a* as relative fluorescence on a log scale (abscissa) versus cell number (ordinate). In *b*, geometric mean fluorescence intensities are given for cells incubated with or without HEL in 5 separate experiments of this type. Per cent of initial level for each experiment was calculated as (mean fluorescence intensity of cells incubated with HEL)/(mean fluorescence intensity of cells incubated without HEL) $\times 100\%$, and is followed by the mean and s.e. for the 5 experiments as a whole. No significant size enlargement of the $A\alpha^kA\beta^k$ positive cells was noted in any of these experiments based on forward scatter.

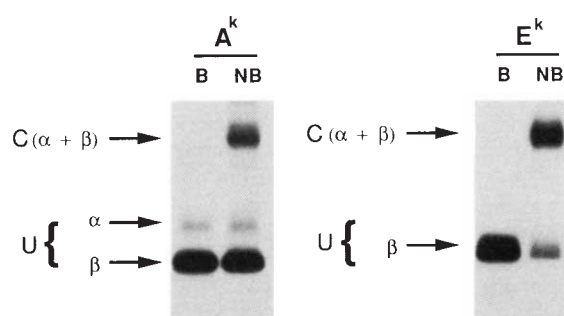


FIG. 6 Unstable class II molecules on the cell surface. Surface proteins on CBA spleen cells were iodinated, the cells lysed, and labelled proteins immunoprecipitated using either anti- $A\beta^k$ antibody (A^k) or anti- $E\alpha$ antibody (E^k). Immunoprecipitated proteins were eluted with heat (B) or at room temperature (NB), then subjected to SDS-PAGE. Labels are as in Fig. 1. Note that murine class II α proteins routinely label poorly with iodine.

METHODS. Freshly isolated CBA spleen cells kept at 4°C were surface-labelled with ^{125}I using lactoperoxidase, as described ref. 64. Labelled cells were lysed and proteins immunoprecipitated, eluted, then subjected to SDS-PAGE and autoradiography as described in the legend to Fig. 1. The absence of labelled internal proteins is evident from the lack of co-precipitated invariant chain or actin.

molecules represent class II dimers with associated peptide. It is unlikely that such association, if it occurs, is similar to the slow dissociation binding state observed for biologically active class II-peptide complexes^{47,51,52}. Peptide binds to free α and β chains, some of which represent dissociated U dimers, and to a partially denatured form of class II dimer ('floppy')⁵³ not observed in these *in vivo* biosynthetic studies. As our data⁶⁵ indicate that any such binding is of substantially lower affinity than with C complexes, peptides bound to U dimers would be rapidly lost from class II during transport and after exposure on the cell surface, again supporting the notion that these U molecules are functionally empty.

C-state molecules were not observed among newly assembled class II dimers in the ER, even using antibodies biased to the detection of this conformation. For $E\alpha^k E\beta^k$, we can estimate that no more than 0.1–0.2% of newly synthesized class II achieves the stable state in the ER. Assuming 2×10^5 class II molecules per B cell and equivalent representation of all peptide-loaded stable molecules on the surface, this would give no more than 200–400 peptide-MHC complexes on the membrane that derive from endogenous peptides loaded in the ER. This number, which is at the lower limit of peptide-MHC complexes needed for T-cell stimulation^{54,55}, would be reached only if a single peptide occupied all class II molecules loaded in this compartment. Nevertheless, there are numerous reports of endogenously synthesized protein being presented by class II MHC molecules without apparent entry into the endosomal/lysosomal pathways^{12–18}. The apparent contradiction between our biochemical results and these functional observations imply that either: (1) our method fails to identify a class of biologically active peptide-class II MHC complexes formed in the ER which is structurally distinct from the complexes formed in acidic compartments from exogenous antigens, even though both are recognized by the same T-cell antigen receptor, or (2) that stable association with class II of peptides derived from endogenously synthesized proteins actually occurs in another (non-ER) site under these conditions. In the former case, one might imagine a loosely associated peptide-class II complex that survives for the time needed to reach the cell surface without deviation by Ii into the normal class II processing pathway.

These studies begin to integrate the process of peptide-binding with the well-described class II biosynthetic pathway and complement ultrastructural studies examining class II and antigen localization^{56–59}. They reveal an important role for peptides in regulating both the lifetime of class II and its surface expression, also seen with class I molecules^{6–10}, and provide *in vivo* evidence for a function of Ii in controlling the site of class II peptide acquisition. Our findings support the view that class I and class II MHC proteins have been selected to possess distinct but highly related structures which mediate effective peptide acquisition in different intracellular environments¹. □

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Is the bulge of our Galaxy triaxial?

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AS in the case of other spiral galaxies, such as M31 (refs 1, 2), the rotation curve of gas in our Galaxy indicates that its nuclear bulge is triaxial³. Despite several studies^{4,5} of the distribution of specific objects in the galactic bulge, no observational evidence of asymmetry has emerged. Recently, however, Blitz and Spergel⁶ have reanalysed balloon-infrared observations at 2.4 μm , where obscuration is relatively low, to suggest that the galactic bulge is bar-like, and tilted with respect to the plane of the Galaxy. Here we report that the distribution of IRAS (the Infrared Astronomy Satellite) bulge stars shows an asymmetry with respect to the Galactic Centre, providing clear evidence for triaxiality.

Because of the large interstellar absorption, the galactic bulge cannot be observed at optical wavelengths, except in some optical windows where the extinction is relatively low⁷. Several infrared observations of stars in the bulge have been made from balloons^{8,9}. Furthermore, Habing *et al.*^{10,11} have shown that the ratio of the infrared flux at 25 μm to that at 12 μm (F_{25}/F_{12}) from the IRAS point source catalogues¹² can be used to delineate the bulge components. Rowan-Robinson and Chester¹³ have constructed a quantitative model for the infrared emission from the galactic bulge based on the IRAS observations. It is known that the IRAS sources with $\log(F_{25}/F_{12}) > -0.5$ are more likely to be associated with the galactic bulge than the galactic disk^{10,11,13}.

To investigate a possible asymmetry in the distribution of bulge stars, we have used the IRAS observations¹². Figure 1 shows a 12- μm number-luminosity relation for the IRAS sources with colours $0 < \log(F_{25}/F_{12}) < 0.1$ and in the region with galactic latitude and longitude $|b| > 3^\circ$ and $|l| < 10^\circ$. We have plotted the 337 sources in the positive (solid line) and negative (broken line) galactic longitudes separately. The colour criterion was imposed to ensure that we selected a narrow range of late-type spectral stars, which are stars with a dust shell of temperature 280 K and luminosity $3 \times 10^3 L_\odot$ in the bulge (when an optically thick dust shell is assumed). If we assume the intrinsic luminosities of these objects are the same, then the abscissa, $F_{12}^{-0.5}$, can be interpolated as a measure of distance from the Sun. Figure 1 shows that there is a systematic shift along the horizontal axis between sources at the positive and negative galactic longitudes. Peaks near $F_{12}^{-0.5} \approx 0.53$ ($F_{12} \approx 3.5$ Jy) and a sharp decline of the source number beyond $F_{12}^{-0.5} \approx 0.58$ ($F_{12} \approx 3$ Jy) are notable. The detection limit for the IRAS sources at 12 μm in this region is ~ 0.5 Jy so that these peaks are well above the detection limit. The stars with $3 \times 10^3 L_\odot$ in our colour criterion should have $F_{12} = 3$ Jy at the Galactic Centre (distance $d = 8$ kpc). Thus we are sampling stars through the galactic bulge and the sampling is complete.

The difference between the two samples corresponds to a systematic flux difference of $\sim 30\%$. The Kolmogorov-Smirnov test shows that the difference is significant at the 97.5% confidence level (the K-S distance between two samples was 0.15, which gave the 2.5% significance for the null hypothesis

that they were selected from the same distribution function). To visualize this asymmetric distribution more clearly, we have plotted the positions (in galactic coordinates) of a subsample of 50 IRAS sources with $0 < \log(F_{25}/F_{12}) < 0.1$ and $6 \text{ Jy} < F_{12} < 11 \text{ Jy}$ (Fig. 2). Sources with low galactic latitude ($|b| < 3^\circ$) are not included in the figure to avoid contamination of the disk components. The asymmetric distribution of the sources with respect to the axis of galactic longitude $l = 0$ is apparent.

We now consider possible explanations for this asymmetric distribution. One can argue that the bulge has a bar-like structure and the major axis lies along a direction from positive to negative longitude in such a way that the near side of the bar is seen at positive galactic longitude. Thus the observed systematic luminosity difference seen in Fig. 1 is due to the distance effect. If we adopt the distance to the Galactic Centre 8 kpc, we obtain average distances of 7.5 and 8.5 kpc for $l > 0$ and $l < 0$ sources, respectively. Unfortunately, the axial ratios of the bulge cannot be determined uniquely from the present analysis. Further detailed analysis of the luminosity function for the well defined sources is necessary to answer this question.

If the above argument is correct, then there is a definite possibility that the galactic bulge is tilted with respect to the galactic disk. Observations of H I and CO lines (for example ref. 14) have indicated that the nuclear disk is tilted by $\sim 22^\circ$. It is difficult to recognize the tilt of the bulge from Fig. 2. The numbers of sources in the four quadrants in Fig. 2 are 6, 16, 19 and 9, respectively. The excess of the sources in the third quadrant in Fig. 2 may be interpreted as meaning that the major axis of the bar-shaped bulge is tilted to the third quadrant (that is, $l > 0$ and $b < 0$). The number of IRAS sources in our sample is rather small, however, and the excess of sources in the third quadrant is marginal.

A second possible explanation for the asymmetry is that the sample we used is contaminated by the disk component. This,

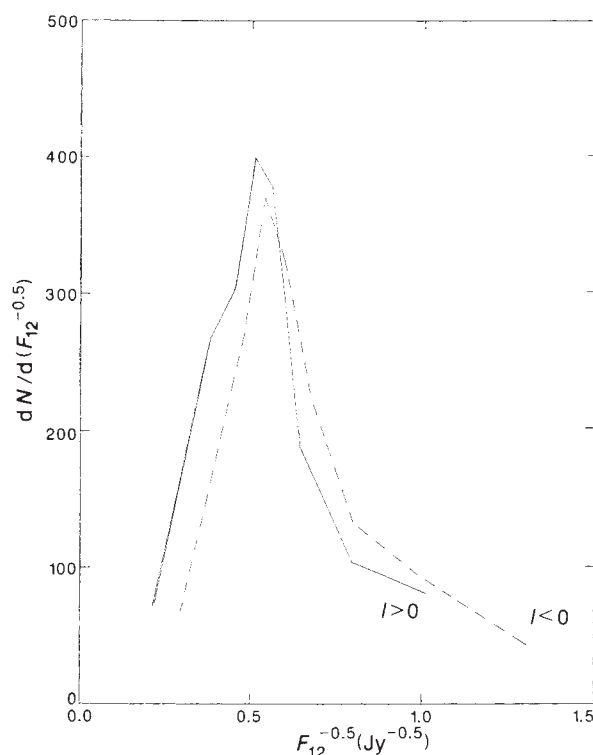


FIG. 1 Number-luminosity plot for the IRAS sources with $0 < \log(F_{25}/F_{12}) < 0.1$, $3^\circ < |b| < 10^\circ$, and $|l| < 10^\circ$. The horizontal axis, $F_{12}^{-0.5}$, is a measure of distance from the Sun when the intrinsic luminosities are the same for all sources. The peaks are close to $0.5 \text{ Jy}^{-0.5}$, which corresponds to a 12 μm flux density of 4 Jy. The almost parallel shift of the number-luminosity relation of $l > 0$ (solid line) to $l < 0$ (broken line) indicates that the $l > 0$ sources are systematically closer to the Sun.

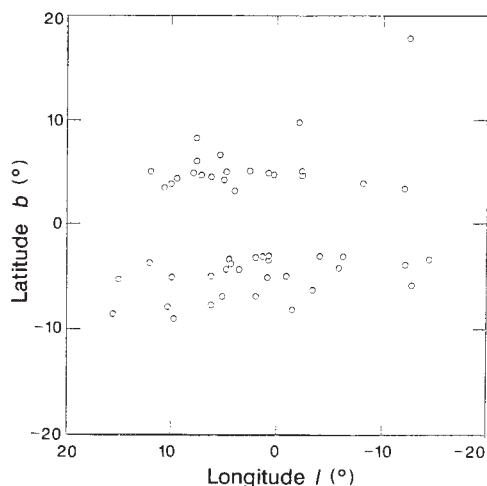


FIG. 2 Map (in galactic coordinates) of IRAS sources with $0 < \log (F_{25}/F_{12}) < 0.1$ and $6 \text{ Jy} < F_{12} < 11 \text{ Jy}$. Sources at low galactic latitude, $|b| < 3^\circ$, are excluded to avoid contamination of the disk component. More of the sources are distributed to the left of the figure ($l > 0$) than to the right ($l < 0$).

however, is unlikely as we excluded sources with $|b| < 3^\circ$, so that only disk sources very close to the Sun (probably with $d < 1 \text{ kpc}$) were included in our sample. Because the extinction is expected to be low, such a nearby star should easily be identified even in optical wavelengths. We have tried to identify such an object in the Palomar Sky Survey, but only 1 out of 20 sources can be found. Therefore, contamination by the disk source cannot explain the observed number difference. Furthermore, the 3.5-Jy peak in Fig. 1 cannot be explained if these sources are nearby objects.

The other possible explanation for the observed asymmetry is that there is an asymmetric distribution of dust absorption toward the direction of the Galactic Centre. It is known from the presence of some optical windows that the dust extinction toward the Galactic Centre is not uniform. It is rather difficult, however, to expect any significant absorption at $12 \mu\text{m}$. As Rieke and Lebofsky¹⁵ suggested that the ratio of absorption at $12 \mu\text{m}$ to that of the K band ($2.2 \mu\text{m}$), A_{12}/A_K , is ~ 0.33 , we can estimate the $12\text{-}\mu\text{m}$ extinction from A_K . According to Hiromoto *et al.*¹⁶, the visual extinction above $|b| > 3^\circ$ is less than 5 mag, which is equivalent to $A_K < 0.4 \text{ mag}$. This is consistent with the estimate of Frogel¹⁷, $A_K = 0.17$ at $b = 3^\circ$. Therefore, the extinction at $12 \mu\text{m}$ is probably less than 0.13 mag at $|b| > 3^\circ$. Furthermore, the systematic differences in the extinction of one side relative to that of the other side must be smaller than the total extinction mentioned above. Thus, we believe that the effect of asymmetric absorption is negligible at 12 and $25 \mu\text{m}$ in the region $|b| > 3^\circ$.

We conclude that the asymmetric distribution of the IRAS bulge sources may indicate that the bulge of our Milky Way Galaxy is bar-like. Our result is consistent with the bar-like bulge proposed by Blitz and Spergel⁶. An alternative model, in which the scale of the bar-like structure is $\sim 4 \text{ kpc}$, has been proposed by Blitz and Spergel¹⁸ from analysis of H I terminal velocities. This is much larger in size, and the orientation inferred for this large bar is almost perpendicular to the bulge bar discussed here. It would be rather intriguing to know how far this non-axisymmetric structure extends. Studies of radial velocities of maser sources in the bulge would contribute a great deal to our understanding of the dynamics of this interesting region of our Galaxy. $\square$

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Compensation of atmospheric optical distortion using a synthetic beacon

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ATMOSPHERIC turbulence limits the resolution of a ground-based astronomical telescope to much worse than the diffraction limit of the instrument. An adaptive-optics system, in which some part of the optical train of the telescope can be adjusted in real time, can compensate for atmospheric turbulence, but a beacon or guide-star is needed to allow measurement of the atmospheric optical distortion. It has been suggested¹ that the measurement could be achieved by means of a synthetic beacon (also called an artificial beacon, and sometimes a laser guide-star) formed by atmospheric backscatter from a ground-based laser. We have performed an experiment to demonstrate atmospheric compensation using a synthetic beacon. With a pulsed dye laser to generate the beacon and a 241-channel adaptive-optics system to perform the phase correction, we obtained almost diffraction-limited resolution of star images in the visible part of the spectrum. Our results indicate that there are no technical barriers to atmospheric compensation using synthetic beacons at ground-based observatories.

The new generation of 8–10 m telescopes will have impressive light-gathering capabilities, but without atmospheric compensation their resolution capabilities will be the same as that for a telescope with a diameter equal to r_0 , the atmospheric coherence length. The effective aperture size ranges from 5–10 cm (1–2 arcsec seeing) for typical turbulence conditions to 20 cm (0.5 arcsec seeing) for the best astronomical sites.

It is well known that atmospheric turbulence can be compensated by using a bright star as a beacon with which to sense the turbulence-induced phase errors². Unfortunately, the requirements that the star should be bright, and close enough in angle to the astronomical object to be within the isoplanatic angle (the angle within which the turbulence is effectively constant) restrict the use of beacon stars. As an example, for adequate spatial and temporal correction at visible wavelengths, a beacon star must have visual magnitude ~ 3 –5, and the isoplanatic angle implies that an object must be within $10 \mu\text{rad}$ of the beacon star to be well compensated. Such requirements obviously restrict this technique to a small portion of the sky. The situation is somewhat better in the infrared, as both r_0 and the isoplanatic angle increase with increasing wavelength; but even in the infrared it is difficult to achieve full sky coverage using star beacons.

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To overcome the limitations of star beacons one may use a synthetic beacon formed by atmospheric backscatter from a ground-based laser. The idea of using a synthetic beacon was apparently conceived simultaneously by several independent groups in the early 1980s; the idea was first reported by Foy and Labeyrie¹. The aim of our experiment was to demonstrate atmospheric compensation using a synthetic beacon.

We performed our experiment using a 60-cm telescope and beam director on the top of Mt Haleakala on the island of Maui, Hawaii. We first tracked a bright star, then set a pulsed laser beam out through the telescope aimed at the star. This laser beam generated a synthetic beacon by Rayleigh backscatter in the atmosphere. The backscattered light was used in a wavefront sensor to measure the turbulence-induced phase aberrations, and these were corrected using a deformable mirror. We diagnosed the degree of atmospheric compensation by looking at the uncorrected and corrected images of the bright star.

Figure 1 shows a schematic illustration of the experimental arrangement. We use a fixed 60-cm telescope followed by a 1-m tracking flat. We track the star by sensing it in a Hamamatsu multi-anode photomultiplier and using the error signals to drive a fast steering mirror at a bandwidth of 225 Hz; the fast steering mirror is in a cascade arrangement with the tracking flat at a bandwidth of 2 Hz. The beacon illuminator laser is a flashlamp-pumped dye laser³ operating at a wavelength, λ , of 508 nm. The laser typically puts out 5–7 J at a repetition rate up to 5 Hz. Because the beam quality of the laser is poor we spatially filter the laser by focusing it through an aperture with angular width $28\lambda/D$, which gives us a beam divergence of $24\ \mu\text{rad}$ out of the telescope ($D=60\text{ cm}$) and typically leaves us with $\sim 2\text{ J}$. The laser beam is inserted into the main optical train, circularly polarized, and transmitted through the 60-cm telescope. The beam is focused at ranges from 4 to 8 km, where it generates a

synthetic beacon by Rayleigh backscatter from atmospheric nitrogen and oxygen. The backscattered light, still circularly polarized, is collected by the 60-cm telescope, linearly polarized by a quarter-wave plate, split off from the outgoing beam by a polarizing beam splitter, and sent into the wavefront sensor. At the entrance to the wavefront sensor, adjustable lenses remove the static focus of the synthetic beacon corresponding to the backscatter altitude. A Pockels cell switches the wavefront sensor on at a time corresponding to a selected altitude between 4 and 8 km. Typically the sensor is gated on for 5–10 μs , corresponding to a synthetic beacon length of 0.75–1.5 km in the sky.

The wavefront sensor is a 16×16 Hartmann-type sensor that we developed especially for this experiment. It measures 218 x and 218 y phase gradients, corresponding to a 16×16 array with the corners cut off to conform to our circular telescope aperture. The sensor uses small-lens arrays fabricated using binary optics, and it has two 64×64 CCD chips⁴, illuminated from behind, as detectors, one to measure x gradients and one to measure y gradients. Each CCD chip has a quantum efficiency of 80% at 508 nm and a noise of less than 30 electrons per pixel at a readout rate of 4.2 megapixels per second. These characteristics permit us to use the bare CCD as a focal plane, with no intensification necessary. The sensor has a dynamic range of ± 1.4 waves in each of the 436 measurement subapertures and an accuracy of $\lambda/25$. The sensor measures and reads out a frame of phase gradients in 225 μs .

The 218 x and 218 y phase gradients from the wavefront sensor are sent to a special-purpose reconstructor⁵, which generates 241 phase values by a matrix-multiplication implementation of a least-squares reconstruction technique. The reconstructor works while the phase gradients are being read out, so no additional time is taken for the calculation.

The 241 phases are used to drive a 241-actuator deformable

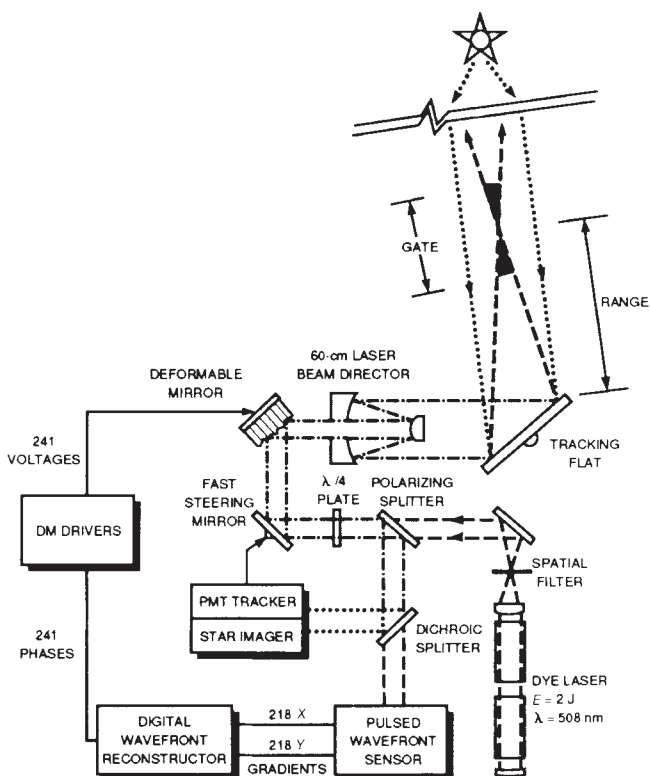


FIG. 1 Experimental arrangement. A bright star is tracked in a 60-cm telescope. A flashlamp-pumped dye laser puts out a 2-J, 508-nm pulse at 5 Hz. The beam is focused at 4–8 km, to generate a synthetic beacon by Rayleigh backscatter from atmospheric nitrogen and oxygen. Using the backscattered light, a wavefront sensor measures the atmospheric phase distortions, and the phase distortions are corrected by a 241-actuator deformable mirror.

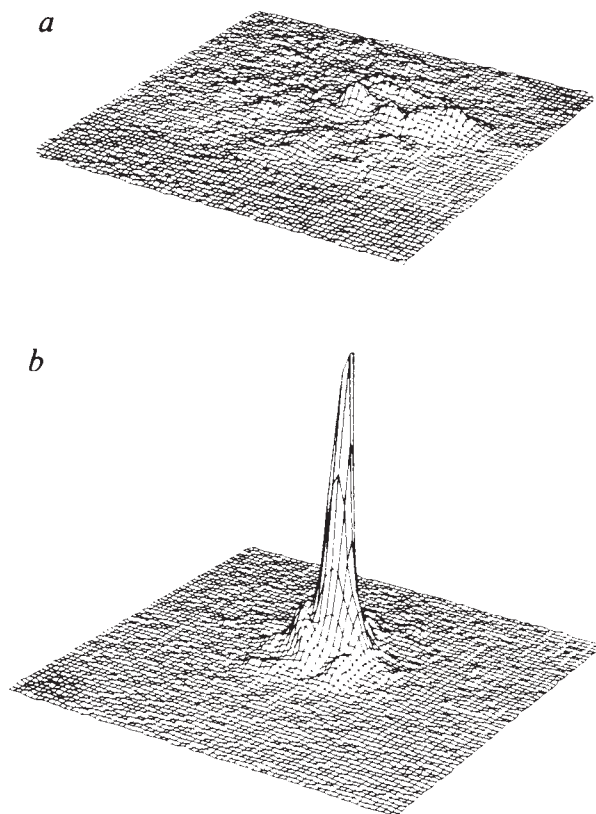


FIG. 2 Images of Procyon at a zenith angle of 20° taken with a 64×64 CCD camera. *a*, Uncompensated star image. *b*, Compensated star image; Strehl ratio is 0.46. CCD camera field of view is $19\ \mu\text{rad}$. Synthetic beacon at 6 km, length 1.5 km. $r_0 = 10\text{ cm}$.

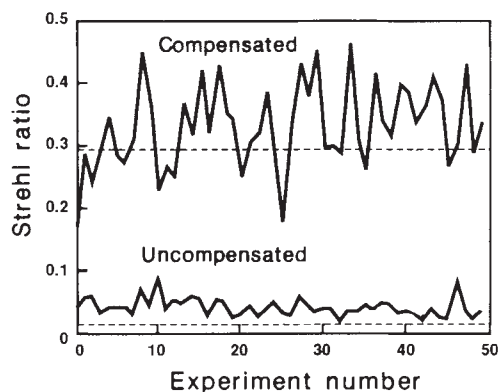


FIG. 3 Strehl ratios for 50 consecutive compensation cycles at an update rate of 2.5 Hz. Synthetic beacon at 6 km, length 1.5 km; $r_0 = 10$ cm. The dotted lines show the Strehl ratios of the long-term averages for the compensated and uncompensated images.

mirror built for us by Itek. The mirror has a continuous facesheet with discrete lead-magnesium-niobate actuators mounted behind. Each actuator can locally deflect the facesheet by $3.5 \mu\text{m}$ when a voltage of 300 V is applied. The complete deformable-mirror system will respond from flat to full applied figure in less than 300 μs . We carefully calibrated the deformable mirror and its drive circuits so that any specified mirror surface can be matched to an accuracy of $\lambda/25$ r.m.s.

The image of the star is measured by a 64×64 CCD focal plane similar to those used in the wavefront sensor. The starlight is filtered so that only radiation in the bands $0.4\text{--}0.5 \mu\text{m}$ and $0.55\text{--}0.6 \mu\text{m}$ is measured.

Because the repetition rate of the dye laser is low compared with atmospheric relaxation rates, we do not perform a conventional closed-loop correction with the adaptive-optics system. Instead, we use an open-loop, 'go-to' technique in which we perform a complete correction on each beacon pulse. This technique allows us to use a lower average laser power, but it requires accurate open-loop calibration of the wavefront sensor and the deformable mirror. A typical correction cycle is as follows. We first flatten the deformable mirror. We then take a 1-ms exposure of the uncompensated star image, and immediately afterwards, fire the dye laser. We then measure the atmospheric phase from the synthetic beacon and command the deformable mirror to apply the conjugate of that phase. The entire process, from the firing of the laser to the deformable mirror achieving its commanded phase, takes only 500 μs . Once the deformable mirror has settled at the correct phase, we take a 1-ms exposure of the compensated star image.

In Fig. 2 we show star images for a representative good synthetic-beacon compensation. The images are of the star Procyon at a zenith angle of 20° . The synthetic beacon for this experiment was centred at 6 km and had a total length of 1.5 km. The measured turbulence coherence length for this night was $r_0 = 10$ cm, and the measured isoplanatic angle was $\sim 20 \mu\text{rad}$. The uncompensated image is ill-formed and smeared; in fact, it slightly exceeds the width of the focal-plane array. The compensated image, in contrast, is a well-formed spot with considerably increased peak intensity. The measured Strehl ratio (ratio of peak intensity to diffraction-limited peak intensity) for the compensated beam is 0.46. The width of the compensated beam at half maximum, which may be taken as the system resolution, is essentially diffraction limited, indicating that the Strehl ratio of 0.46 results from energy thrown into the wings, not from a spreading of the central lobe.

In Fig. 3, we plot compensated and uncompensated single-frame Strehl ratios for a series of 50 consecutive correction cycles taken at a rate of 2.5 Hz. We also show the Strehl ratios of the long-term-average compensated and uncompensated images. The Strehl ratio of the long-term-average uncompensated image is 0.02, less than half the average of the individual uncompensated Strehl ratios, 0.05. The reason for this disparity is that the uncompensated images are broken up and lack a central core; averaging multiple images results in a

broad smear of energy across the focal plane rather than in reinforcement of a central peak. As one would expect for well-compensated images, the Strehl ratio of the average compensated image is comparable to the average of the individual Strehl ratios (~ 0.3).

We have analysed the compensated Strehl ratios and have found them to be in good agreement with those expected from known error sources. The principal errors are sensor noise, because the signal level is marginal, and focal anisoplanatism, the error caused by the fact that the ray paths for the synthetic beacon focused in the atmosphere do not exactly correspond to the essentially collimated ray paths from the star.

We have performed atmospheric-compensation experiments with synthetic beacons under many atmospheric conditions and have varied parameters such as the beacon altitude, the zenith angle and the offset angle between the beacon and the star. In addition, we have made concurrent measurements of atmospheric turbulence. We will report detailed analysis of the results elsewhere (manuscript in preparation).

Our experimental results demonstrate that atmospheric compensation can be successfully performed using synthetic beacons. These results were obtained using a relatively low-altitude synthetic beacon formed by Rayleigh backscatter. In related activities at MIT Lincoln Laboratory, phase measurements have been made using a synthetic beacon formed in the mesospheric sodium layer at 90 km (ref. 6) and solid-state laser systems for accessing the 589-nm sodium resonance line have been developed⁷. Atmospheric compensation systems using sodium beacons should be superior for astronomical applications, but they have not yet been implemented because the required energy, bandwidth and pulse length make the illuminator laser difficult to fabricate. The combination of our experimental results and the work described in refs 6 and 7, however, gives us confidence that an atmospheric-compensation system using a sodium beacon could be developed in the near future and that such a system could achieve near-diffraction-limited imaging in the visible for a large astronomical telescope. $\square$

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Measurement of atmospheric wavefront distortion using scattered light from a laser guide-star

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THE possibility of using an artificial 'guide-star' to measure optical wavefront distortion caused by atmospheric turbulence has been discussed for some time¹⁻⁴, but few experimental data are available⁵. Here we report experimental results demonstrating that atmospheric wavefront distortion can be measured by taking fast 'snapshots' of a guide-star formed by light scattered from a laser beam focused in the upper atmosphere. These results agree with a theoretical prediction⁶ that the mean-square wavefront error created by using an artificial guide-star at a finite distance rather than a real (infinitely distant) star is proportional to the five-thirds power of the telescope aperture. Using this understanding of the physics, we have demonstrated continuous, real-time atmospheric compensation of a 1.5-m telescope using a high-pulse-rate laser, pulse-synchronized wavefront sensor and deformable mirror, and have been able to resolve the 1.3-arcsecond binary star 53 ξ Ursa Majoris in an exposure time of only one second.

The US Department of Defense has been developing laser guide-star adaptive optics since October 1981, but has allowed public release of information only since May 1991. The programme started following a suggestion made by Julius Feinleib of Adaptive Optics Associates for creating artificial stars from the Rayleigh scattering of a laser beam focused in the upper atmosphere. Because rays from the laser guide-star beacon may

travel along a different-path from those of the star under observation, the effects of atmospheric turbulence may be quite different in each case, resulting in focus anisoplanatism. Fried assessed the usefulness of such a finite-range guide-star, and showed that useful performance could be achieved even with the wavefront-distortion error caused by focus anisoplanatism. In 1982, laser excitation of mesospheric sodium was suggested⁹ as a means of generating artificial guide-stars at a significantly higher altitude to reduce the focus anisoplanatic error. The Defense Advanced Research Projects Agency (DARPA) sponsored two experiments—one to test the Rayleigh scattering idea and the other to test the resonant mesospheric sodium concept. Here we report the results of the Rayleigh guide-star experiment conducted at the Starfire Optical Range (SOR) in New Mexico during 1983. The sodium experiment was conducted at White Sands Missile Range, New Mexico, during 1984¹⁰. In 1985, independently of the work of the Department of Defense, the idea of using the Rayleigh and sodium laser guide-stars concepts was suggested¹. Thompson and Gardner⁵ generated a mesospheric sodium guide-star and verified radiometric predictions. In mid-1988, the Massachusetts Institute of Technology Lincoln Laboratory showed atmospheric compensation on a 0.6-m aperture using a Rayleigh laser guide-star operating at a few pulses per second and a special 'go-to' control system for their adaptive optics system. In February 1989, our group at Phillips Laboratory demonstrated high-bandwidth, closed-loop atmospheric compensation on the SOR 1.5-m telescope using a high-repetition-rate Rayleigh laser-guide-star. We analysed stellar images formed at an average wavelength of $0.88 \pm 0.05 \mu\text{m}$ while operating the adaptive optics at a closed-loop bandwidth of 30–65 Hz using a laser guide-star at a range of 10 km. We achieved Strehl ratios (ratio of actual peak intensity to diffraction limited peak intensity) of >0.2 , full-width-half-maximum resolution of 0.18 arcsec, and image intensity improvements of nearly an order of magnitude in the presence of visible wavelength atmospheric seeing conditions of 2 arcsec (R.Q.F. *et al.* manuscript in preparation). Figure 1 shows the improvement in a binary star image achieved with the laser guide-star adaptive optics operating on the 1.5-m telescope.

The primary objective of our first laser guide-star experiment performed in 1983 was to determine whether a laser beam focused in the atmosphere really could be used as a beacon to

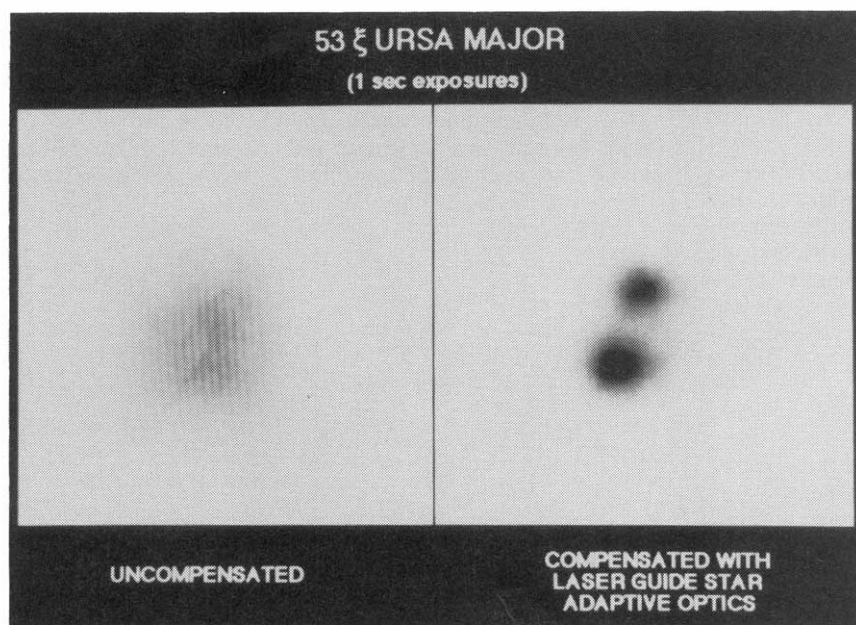


FIG. 1 Charge-coupled-device camera images of the binary star 53 ξ Ursa Majoris ($m_v=4.2$ and 4.5, separation ~ 1.3 arcsec) made through the SOR 1.5-m telescope. Each image is 6.24 arcsec square exposed for one second at an average wavelength of $0.88 \mu\text{m}$ and spectral bandwidth of $0.1 \mu\text{m}$. The laser guide-star adaptive optics was operated at 65-Hz closed-loop bandwidth. The peak intensity in the compensated image is 8.6 times the peak intensity in the uncompensated image. The Strehl ratio of the compensated image is ~ 0.1 and the full-width-half-maximum size of the bright star image is 0.48 arcsec. Full-width-half-maximum image sizes of <0.2 arcsec were achieved for exposures of ~ 20 ms. The degradation at long exposures is due largely to imperfect overall tilt compensation provided by a separate tracker and steering mirror.

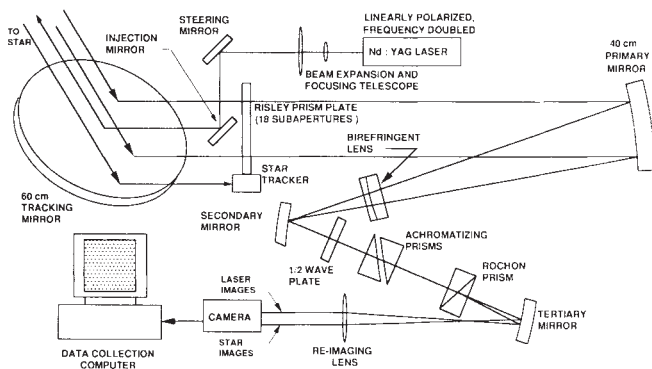


FIG. 2 Schematic diagram of the experimental set-up used for Rayleigh laser guide-star measurements of atmospheric-turbulence-induced wavefront distortion. With the exception of the 60-cm tracking mirror, all components were mounted on a large optics table. The 40-cm-diameter wavefront-distortion-sensing telescope consisted of an off-axis paraboloidal primary mirror and a convex secondary mirror. Hartmann sensor subapertures for measurement of wavefront distortion were defined by 18 Risley prisms mounted in a large plate in front of the 40-cm primary mirror. The subapertures were arranged on two concentric circles with 12 on the outer ring and six on the inner ring. The telescope optics focused all the light on a split photocathode intensifier coupled to a charge-injection-device sensor array. Because of the different settings of each of the Risley prisms, 18 distinct images of a point source were formed. The set of 18 associated centroids defined the wavefront tilt at each of the subapertures. The birefringent lens compensated for the difference in focus between the laser and star beacons.

measure atmospheric-turbulence-induced wavefront distortion, and to assess the quality of the measurement values it produced. The reference (or 'truth') results for this experiment were provided by simultaneous wavefront-distortion measurements using a star as the beacon. Wavefronts determined using the laser beacon were compared with simultaneous wavefront-distortion measurements made using on-axis starlight, the r.m.s. discrepancy between the two wavefronts being compared with theory. The optics were arranged so as to direct the laser beam within a few microradians of the star, thereby, ensuring that light from both the star and the laser guide-star beacons propagate through nearly identical turbulence.

Figure 2 shows the essential features of the experimental set-up. The tracking mirror was positioned by a two-axis gimbaled mount under the control of a low-bandwidth star tracker which kept the optical axis of the 40-cm phase-measurement telescope within one microradian of the star's average position. We used the star Polaris for these experiments because the two-axis gimbal was limited to $\pm 1^\circ$ of total motion in each axis. The 300 mJ per pulse, Q-switched laser beam which we used was expanded to 10-cm diameter, in nearly collimated form, and directed out of the centre of the 40-cm wavefront-sensing aperture, and aligned to be parallel to its optical axis. The beam was focused at a range of 5 km for most tests and the sensor was range-gated to accept backscattered light from 4.5 to 5.5 km.

The wavefront sensor consisted of an 18 subaperture Hartmann-type arrangement, each subaperture defined by an 8-cm

diameter Risley prism. The subaperture geometry consisted of two concentric circles with 12 subapertures on the outer circle and six on the inner circle. Each subaperture produced its own distinct image of a beacon, the location of each of the images being determined by the setting of the corresponding Risley prism. The prisms were rotated in their mounts to provide adequate space between the 18 subaperture images formed at the focal plane of the 40-cm diameter telescope. Two distinct sets of 18 such images were formed, one set being images of the laser guide-star and the other being images of the star.

To measure wavefront distortion simultaneously with the star and laser beacons, light from the two sources, after passing through the subapertures, was spatially separated by polarization, using the Rochon prism. This light, now sufficiently divided so as to be able to form two distinct sets of 18 spots, was imaged onto separate halves of a specially made, independently gated, split photocathode intensifier coupled to a charge-injection-device sensor array. All the light from the laser return was directed to one photocathode (where 18 suitably separated, laser spot images were formed) as the Rayleigh backscatter process does not depolarize the linearly polarized outgoing laser beam. The starlight was equally divided by the Rochon prism between the two halves of the photocathode as this light is randomly polarized—but the starlight going to the laser half of the photocathode was in effect neglected because of the shortness of the time for which that part of the photocathode was on. The laser-range gate was on for only a few microseconds,

FIG. 3 Representative sample of measured wavefront distortions and distortion difference. The lower-left plot shows the wavefront distortion measured using the starlight whereas the lower-right plot shows the wavefront distortion measured using the laser light—the Rayleigh laser guide-star. These distortions have mean square values of 4.434 rad^2 and 4.236 rad^2 respectively. The centre plot shows the difference between these two wavefronts. Its mean-square value of 0.363 rad^2 is a measure of the magnitude of the focus anisoplanatism—in this case for a 40 cm diameter aperture, viewing at a 35° elevation angle, using a guide-star at 5 km range, and with wavefronts corresponding to a wavelength of $0.53 \text{ } \mu\text{m}$.

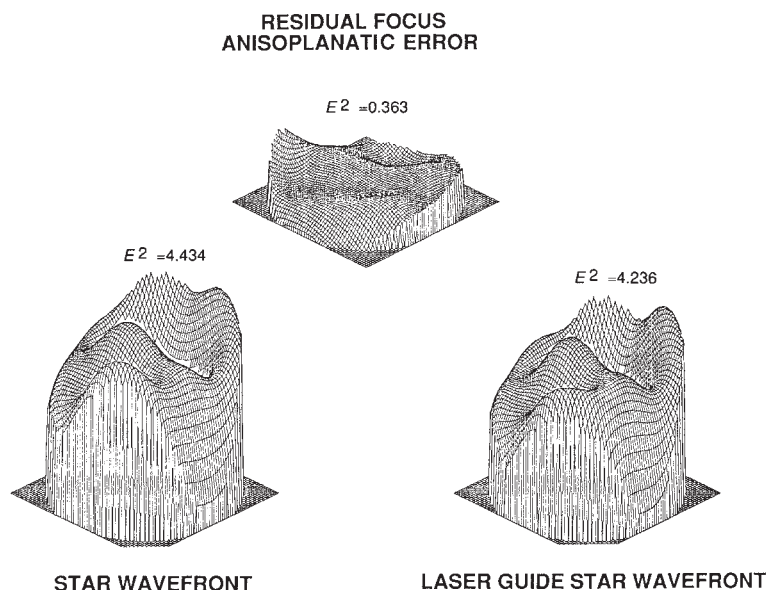
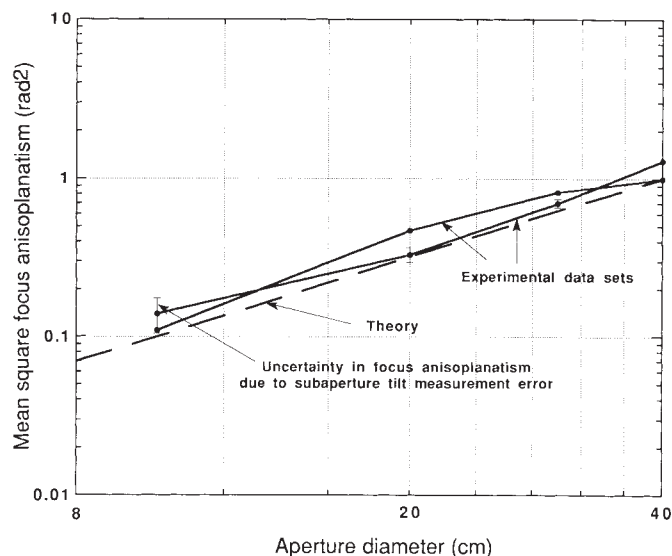


FIG. 4 Mean square focus anisoplanatism error. The term focus anisoplanatism refers to the fact that wavefront distortion measured with a laser guide-star will be somewhat different from what is needed to correct the turbulence induced wavefront distortion of light from a co-aligned star or other source more distant than the laser guide-star. When a laser guide-star is used to control an astronomical adaptive optics system, the focus anisoplanatism will result in a wavefront correction error. We show here the measured focus anisoplanatism for viewing a laser guide-star at 35° elevation and 5 km range, the error being expressed in radians² for a wavelength of 0.53 μ m. The points shown represent two sets of data each formed by averaging over 35 wavefront distortion measurements. The broken straight line represents a theoretical prediction [cf. equation (1)] for which $d_0 = 0.39$ m. The measurement data not only shows the predicted five-thirds power dependence on aperture diameter, but also lies surprisingly close to the $d_0 = 0.39$ m theoretical curve. The uncertainty in our measurement of focus anisoplanatism is indicated by the error bars associated with one of the data sets. These errors were computed using a theoretical treatment of induced mean-square error associated with the difference of two reconstructed wavefronts [15, 16]. This theory utilizes the rms subaperture tilt measurement error—experimentally determined for this experiment to be 650 nanoradians by comparison of identical wavefront exposures (i.e. star-star and laser-laser) on each half of the split photocathode sensor. The subaperture tilt error was dominated by sensor noise and the fact that the size of the laser-guide-star images were somewhat larger than the diffraction limit.



whereas the starlight gate had to be on for a few milliseconds in order to collect enough light. The starlight gate 'on' period was centred in time on the laser range gate (but was off during the laser firing period). Using this timing and polarization arrangement, only starlight images were formed on one side of the split photocathode, and only laser light images on the other. The birefringent lens compensated the focus for the difference in range to the laser and to the star beacons. A desktop computer and custom-made timing electronics controlled the laser firing, the individual photocathode gating, and the digital data collection by a frame grabber attached to the charge-injection-device sensor.

We processed many frames of raw image data to determine, for each, the centroid position of each spot and thus the tilts (wavefront gradients) for each subaperture. The gradient data were used to reconstruct the wavefront distortion over the 40-cm aperture, once for the laser beacon data and once for the star data. Figure 3 is a representative example of the wavefront distortion as determined for the star (truth) and for the laser beacon; also shown is the difference between these two, corresponding to what would be the residual error in an adaptive-optics-corrected star wavefront if the laser-beacon data had been used to control the deformable mirror used in the adaptive optics set-up. These results demonstrate qualitatively that laser guide-star beacons are effective in measuring atmospheric-turbulence-induced wavefront distortion.

Theoretical analysis carried out before this experiment predicted that the mean square difference between the tilt-removed laser-beacon wavefront distortion and the star-beacon wavefront distortion should increase with aperture diameter to the five-thirds power⁶. According to theory, a constant of proportionality, d_0 , was defined in such a way that

$$\langle E^2 \rangle = (D/d_0)^{5/3} \quad (1)$$

where D is the aperture diameter and $\langle E^2 \rangle$ is the aperture-averaged square phase difference between the star wavefront and the laser-beacon wavefront, ensemble averaged over turbulence realizations. The parameter d_0 depends on the wavelength of interest, the height of the laser beacon above the ground, and the height dependence of the index of refraction structure function, C_N^2 . Physically, one can think of d_0 as the size of a real aperture for which the wavefront

error incurred by using the laser guide-star would be one radian (r.m.s.).

Equation (1) represents the mean square error incurred by using the laser guide-star beacon when a real guide-star beacon is not available for use by an adaptive optics system. The error results from the fact that the two sources are at different ranges so rays from the two sources follow different paths through the atmosphere to each point in the aperture. Consequently, at the edge of the aperture, rays from the laser beacon sample the atmospheric turbulence along a (potentially significantly) different path than do rays from the star. Figure 4 shows $\langle E^2 \rangle$ derived from the experimental measurements, as a function of aperture diameter¹³. The experimental data are in excellent agreement with the theory predicting a $D^{5/3}$ dependence.

The value of d_0 is easily calculated from the data in Fig. 4 and equation (1) as being 0.39 m. The theoretical estimate of d_0 for our wavelength, zenith angle and backscatter altitude, using a nighttime turbulence profile model is 0.39 m¹⁴. Experiments performed in more benign atmospheric conditions and with a range gate at 15 km, produced mean-square-wavefront errors as low as 0.18 rad² and a d_0 of 1.1 m, which is good enough to allow wavefront correction to 1/15 of a wave over our 40-cm aperture. □

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Crystal structure and bonding of ordered C₆₀

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STRUCTURAL studies¹⁻³ of crystalline C₆₀ (ref. 4) have indicated that at room temperature the C₆₀ molecules are orientationally disordered and the crystal structure may be regarded as a face-centred cubic configuration of C₆₀ spheres. Below 249 K, however, the molecules become orientationally ordered³ and a simple cubic lattice results, corresponding to a symmetry change from *Fm* $\bar{3}$ to *Pa* $\bar{3}$. Here we present the results of a neutron powder diffraction study of the low-temperature ordered structure, which reveals the packing configuration of the C₆₀ molecules. The C₆₀ units are rotated in an anticlockwise manner around the [111] direction by $\sim 98^\circ$ from the ideal *Fm* $\bar{3}$ configuration. This apparently arbitrary rotation in fact results from an optimized ordering scheme in which electron-rich short (1.391-Å) inter-pentagon bonds face the electron-poor pentagon centres of adjacent C₆₀ units. The high symmetry of the C₆₀ molecule allows these interactions to be optimized identically for all twelve nearest neighbours, a possibility that is by no means intuitively obvious. The bonds common to a given pentagon are somewhat longer (1.455 Å). The high degree of bonding optimization and the absence of bonding frustration accounts for the high ordering temperature of 249 K (ref. 5).

Pure C₆₀ was prepared and purified using standard procedures⁴. Traces of solvent were removed by heating at 170 °C under reduced pressure (0.5 mm Hg) for 2 h. The sample was characterized by X-ray powder diffraction, and infrared and ¹³C NMR spectroscopy. Neutron powder diffraction data were collected at 5 K on the high-resolution powder diffractometer at ISIS for 3 h. The illuminated sample volume was 0.3 cm³ and the data extended from $d = 0.83$ Å to $d = 2.28$ Å ($38 \leq h^2 + k^2 + l^2 \leq 286$). The initial starting model for profile refinement assumed the ideal truncated icosahedral symmetry consistent with space group *Fm* $\bar{3}$. This ideal configuration has atoms located at $\alpha(0, \pm\frac{1}{2}, \pm\frac{1}{2}(2\tau))\text{C}$, $\alpha(\pm\frac{1}{2}\tau, \pm 1, \pm\frac{1}{2}(2\tau+1))\text{C}$ and $\alpha(\pm\tau, \pm\frac{1}{2}, \pm\frac{1}{2}(2+\tau))\text{C}$, where C implies cyclic permutation of coordinates, τ is the golden ratio $1.61083 = (1 + \sqrt{5})/2$ and α is the ratio of the average C-C distance to cubic lattice constant; α is ~ 0.1 and was taken to be so in the initial stages of refinement.

The truncated icosahedron consists of 12 pentagons and 20 hexagons. Here we use the notation 6:6 and 6:5 bonds to denote C-C bonds fusing two hexagons and a hexagon and pentagon, respectively. The reduction of symmetry from *Fm* $\bar{3}$ to a simple cubic space group permits only two possibilities, *Ph* $\bar{3}$ or *Pa* $\bar{3}$. In both cases the lowering of symmetry results from an arbitrary rotation of the C₆₀ unit about the [111] direction. Accordingly, the C₆₀ molecule was rigidly rotated anticlockwise about the [111] direction in 5° steps from $\phi = 0^\circ$ to 120° for both possible space groups. Only the scale factor was varied in each of these refinements. The χ^2 values obtained in each of these 50 refinements are plotted in Fig. 1a, which clearly indicates that *Pa* $\bar{3}$ is the correct space group and that $\phi \approx 98^\circ$. Ideal coordinates corresponding to this particular rotation were used as starting parameters in the subsequent refinements, in which all positional parameters were varied subject to restraints on the pentagon (108.0 (1)°) and hexagon (120.0 (1)°) angles. Refined atomic positions are listed in Table 1. The observed and calcu-

TABLE 1 Structural parameters from Rietveld analysis of C₆₀ at 5 K

Atom	x	y	z
C11	0.2294 (3)	-0.0325 (2)	0.1010 (3)
C12	0.2467 (3)	-0.0540 (2)	0.0061 (2)
C21	0.2081 (3)	0.0646 (2)	0.1289 (3)
C22	0.2066 (3)	-0.1401 (2)	-0.0360 (2)
C23	0.1710 (2)	-0.0963 (2)	0.1590 (3)
C34	0.2236 (3)	0.1122 (3)	-0.0371 (2)
C24	0.2439 (3)	0.0192 (3)	-0.0636 (2)
C31	0.2053 (3)	0.1349 (3)	0.0616 (2)
C32	0.1503 (3)	-0.2017 (3)	0.0202 (2)
C33	0.1323 (3)	-0.1793 (2)	0.1186 (2)

The crystallographic space group is *Pa* $\bar{3}$ (*T*_h⁶). Cubic lattice parameter is 14.04078 (10) Å. The thermal motion was handled as an overall temperature factor of 0.3 Å². The high *m* $\bar{3}$ *m* ideal molecular symmetry is evident from pseudosymmetry relationships between atomic coordinates. For the atom pairs {1, 2} = {C11, C34}, {C12, C24} and {C21, C31}, $x_1 \approx x_2$, $y_1 \approx z_2$ and $z_1 \approx y_2$. For {C22, C32}, $x_1 \approx -y_2$, $y_2 \approx -x_1$ and $z_1 \approx -z_2$, and for {C23, C33}, $x_1 \approx z_1$, $x_2 \approx z_2$. The *R*-factors are *R*_{wp} = 2.3%, *R*_E = 1.6%; *R*_I = 12.9%.

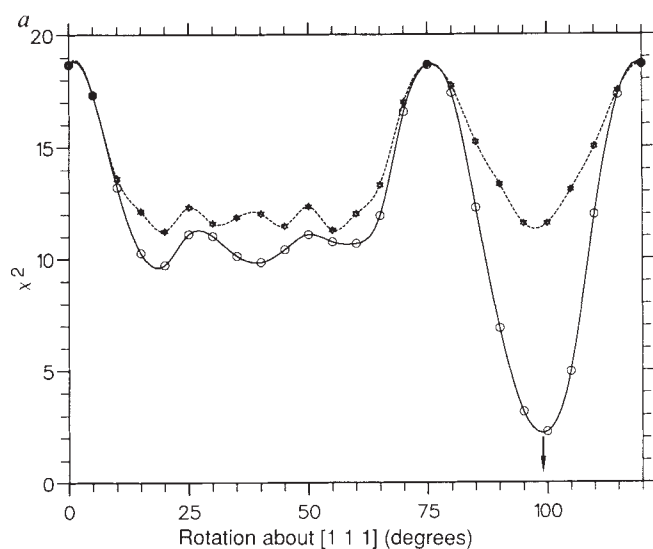
lated diffraction patterns agree well (see Fig. 1b and c). The bond lengths obtained from the Rietveld refinement could clearly be divided into five short 6:6 bonds (1.391 (18) Å) and ten long 6:5 bonds (1.455 (12) Å). (There is some indication that the ten long 6:5 bonds further divide into two sets of 1.444 (18) Å and 1.466 (18) Å, the possible explanation of which is discussed later.) The different 6:6 and 6:5 bond lengths are not unexpected. *Ab initio* calculations⁵ suggest lengths of ~ 1.39 and ~ 1.45 Å. Recent structural work on OsO₄ (ref. 6) and zero-valent Pt (ref. 7) adducts of C₆₀ gives 6:6 lengths of 1.388 (9) and 1.388 (30) Å, and 6:5 lengths of 1.432 (5) and 1.445 (30) Å, respectively. NMR measurements⁸ also discriminate two different bond lengths of 1.400 (15) and 1.450 (15) Å.

The refined crystal structure is illustrated in Fig. 2a. Consideration of the C₆₀ unit centred at the origin shows that the apparently arbitrary rotation of 98° around the [111] has resulted in three pentagons roughly oriented along the [110], [101] and [011] directions. In fact this is not precisely possible; the pentagons must orient along $[\delta 11]$, $[\delta 1\bar{1}]$ and $[\delta 11]$ directions where $\delta = -0.05218$. The corresponding ideal ϕ value is 97.621° , which is extremely close to our observed value. This orientation is not fortuitous. Consideration of the nearest-neighbour C₆₀ units indicates that this leads to a pentagon of one C₆₀ unit facing a 6:6 bond of an adjacent C₆₀ unit. Indeed, the alignment of the pentagons and 6:6 bonds obtained in our refinement is remarkable (see Fig. 2b and c). The 6:6 bond sits directly above the middle of the pentagon face; the dihedral angle defined by (C22-C23) and (C11-C31) (see Fig. 2b) is 179.6° . Given the almost spherical C₆₀ geometry and the fact that all C₆₀ units are symmetry-equivalent, ordering in pure C₆₀ is at first sight surprising. But our observation of two different bond lengths confirms *ab initio* calculations⁵ that the C₆₀ unit has a significantly anisotropic electronic distribution. The pentagons, consisting of

TABLE 2 Intra-molecular bond-lengths (Å) in C₆₀ at 5 K

6:6 bonds		'Non-facing' pentagon		'Facing' pentagon	
C11-C12	1.387	C12-C22	1.459	C11-C21	1.451
C21-C31	1.366	C12-C24	1.420	C11-C23	1.463
C22-C32	1.412	C32-C33	1.439	C21-C31'	1.487
C23-C33	1.405	C24-C32	1.462	C23-C34'	1.482
C24-C34	1.387	C22-C33'	1.439	C31'-C34'	1.445
Average	1.391	Average	1.444	Average	1.466

All standard deviations are ~ 0.010 Å. In the Rietveld refinement the bond lengths were divided into three sets; each set was weakly restrained to be equivalent within ± 0.02 Å without affecting the χ^2 values. The 12 pentagons in the C₆₀ unit may be divided into two types: six that face adjacent C₆₀ units and six that do not.



long bonds, constitute an electron-poor region. The 6:6 bonds exocyclic to the pentagons are short and of higher bond order with significant π -electron density. It is across these bonds that C_{60} reacts with both OsO_4 and Pt^0 . When the C_{60} units are close-packed, the repulsive electrostatic interactions are optimized by having no carbon atom situated above any other carbon atom of a neighbouring C_{60} unit. Furthermore, the electron-rich 6:6 bonds are aligned optimally over the electron-deficient pentagons. As a consequence of the icosahedral symmetry, all twelve nearest-neighbour interactions may be simultaneously optimized. The C_{60} - C_{60} orientational ordering is fully characterized by just one packing motif, illustrated in Fig. 2b. Each C_{60} unit has six electron-deficient pentagons and six electron-rich 6:6 bonds facing its 12 nearest neighbours. It is apparent from Table 2 that two sets of 6:5 bond lengths may tentatively be distinguished. The longer set is associated with the pentagon that faces a neighbouring C_{60} unit. Similarly, although this is barely statistically significant, it is intriguing to note that the 6:6 bond facing the neighbouring C_{60} pentagon is the longest

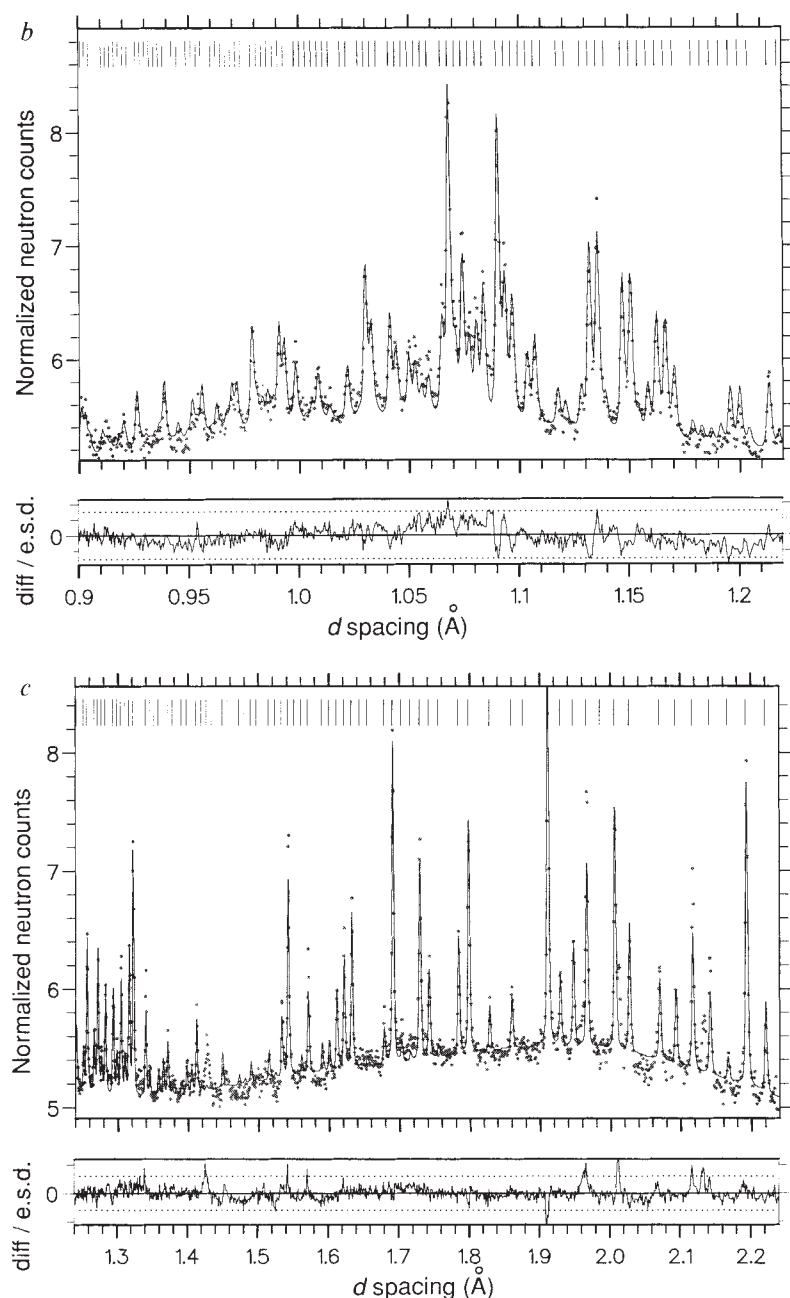


FIG. 1 *a*, Variation of χ^2 as a function of anticlockwise rotation about $[111]$ for space groups $Pa\bar{3}$ ($\circ$) and $Pn\bar{3}$ ($\star$). Angles of 0° and 120° correspond to the ideal $Fm\bar{3}$ configuration. The minimum χ^2 is found for space group $Pa\bar{3}$ at around 98° . *b*, Final observed (dotted) and calculated (solid line) diffraction pattern for C_{60} at 5 K in the region $0.9 \text{ \AA} < d < 1.22 \text{ \AA}$. The dotted lines in the lower plot of difference/estimated standard deviation indicate $\pm 3\sigma$. *c*, as *b* but with $1.24 \text{ \AA} < d < 2.24 \text{ \AA}$.

bond with the lowest bond order. The weak electrostatic repulsion of facing pentagons and 6:6 bonds along the $\langle 110 \rangle$ directions should lead to a small build-up of electron density along the directions farthest away from $\langle 110 \rangle$, namely $\langle 100 \rangle$ directions. This results in an increase in bond order and shortening of bond length in these regions. This is confirmed by our measurements; the shortest 6:5 bond (C12–C24) is 1.420 Å and straddles $\langle 100 \rangle$. The bonding between C_{60} units, although weak, seems to produce appreciable geometrical deviations from $m\bar{3}m$ to $\bar{3}$ point symmetry.

Several authors^{2,9} have pointed out the importance of a clear understanding of the nature of orientationally ordered phases in pure fullerenes. The ordered structure of the prototypical fullerene, C_{60} , determined here, and our detailed explanation of the nature of the bonding that produces the orientational order, are at variance with previous theoretical calculations⁹. Although the emphasis in C_{60} research has shifted to the search for high-temperature superconductivity in alkali metal-doped fullerenes, a detailed knowledge of the ordered C_{60} crystal structure would improve understanding of the properties of fullerenes and fullerites. The combination of the icosahedral structure of the C_{60} unit and the extended simple cubic ordering in solid C_{60} is an elegant example of crystalline symmetry. □

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Isolation of C_{76} , a chiral (D_2) allotrope of carbon

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A LONG search for molecular allotropic forms of carbon^{1–4} culminated in the discovery^{5–7} of a method for preparing large quantities of the C_{60} molecule and the subsequent confirmation^{8,9} of its cage-like truncated-icosahedral structure¹. The C_{70} molecule prepared by the same method was later also isolated and found to have the predicted cylindrical (D_{5h}) structure. Incomplete chromatographic separation of the large molecules C_{76} , C_{78} and C_{84} was achieved at the same time^{10,11}, and small quantities of highly enriched samples were later isolated¹². Preliminary NMR and spectroscopic studies of these carbon clusters failed, however, to provide evidence for the symmetrical structures predicted earlier¹³. Here we report the isolation and characterization of the C_{76} molecule, a third molecular form of carbon following C_{60} and C_{70} . We isolated and purified substantial quantities of this species using the extraction technique of ref. 6 together with chromatography. The ¹³C NMR spectrum consists of 19 lines of essentially equal intensity, confirming a compact, cage-like fullerene structure. Among the several thousand possible structures for C_{76} , theoretical calculations by Manolopoulos¹⁴ predict a chiral structure with D_2 symmetry, consisting of a spiralling, double-helical arrangement of edge-sharing pentagons and hexagons. Our NMR spectrum is uniquely consistent with this remarkable structure.

We isolated C_{76} following methods previously described, in a three-step procedure. (1) The crude toluene-soluble material is separated chromatographically into the fractions C_{60} , C_{70} (with residual C_{60}), and higher fullerenes (with residual C_{70}). (2) After 100 mg of higher fullerenes have been collected, they are separated on an alumina column with hexane/toluene elution, to yield three fractions: (I), C_{70} , (II), C_{76} and C_{78} (with a little C_{70} and C_{84}); and (III), the major component C_{84} (with C_{76} , C_{78} and C_{70}), thus isolating 30 mg C_{76} containing C_{78} as

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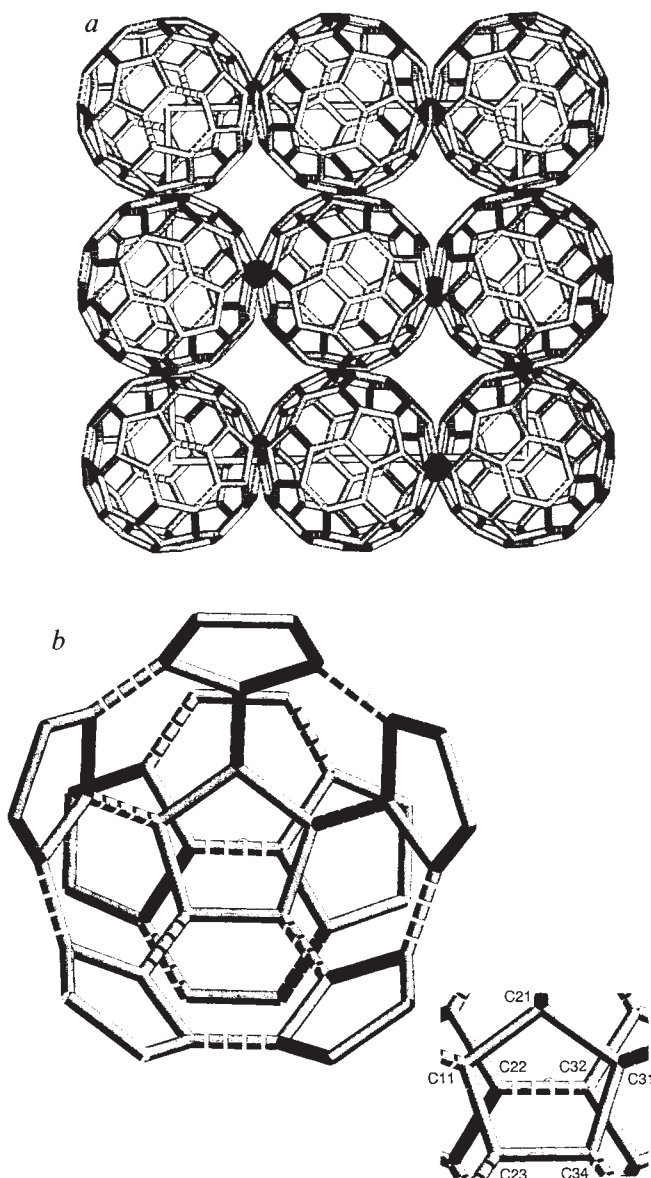
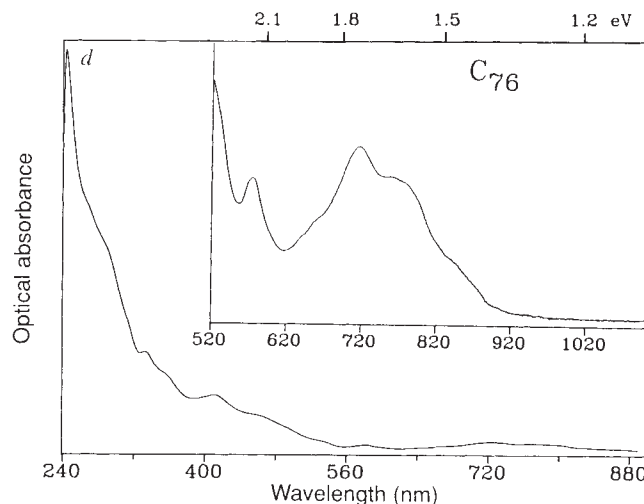
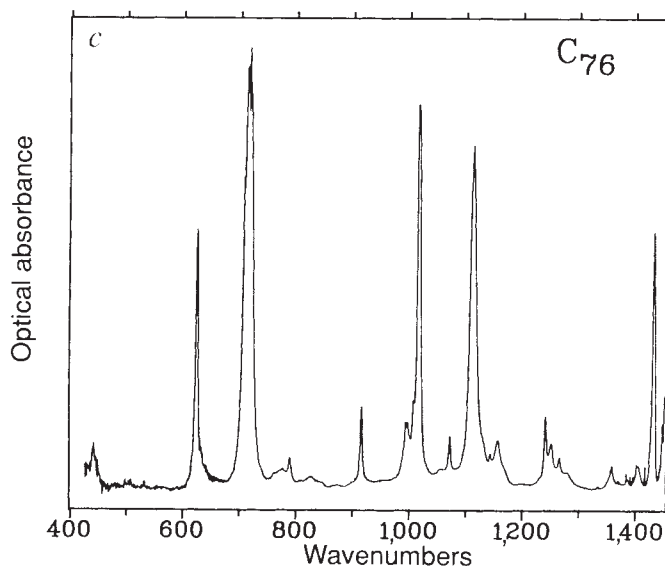


FIG. 2 a, Projection along $[001]$ of the ordered C_{60} structure at 5 K. The hatched bonds are the short 6:6 bonds separating hexagons. The solid bonds are the long 6:5 bonds defining the pentagons. b, Projection of two C_{60} units along $[110]$, the direction from C_{60} centre to C_{60} centre. Only fragments of the C_{60} units are shown for clarity. The 6:6 bond of the neighbouring unit is located in this projection in the centre of the pentagon face. This configuration minimizes C–C overlap in neighbouring C_{60} units and also brings donor and acceptor regions of adjacent C_{60} units into contact. The bonding between C_{60} units is substantially weaker and longer than within the C_{60} unit itself. This is reminiscent of graphite. Although the average C–C distance is slightly longer (3.44 Å) than in graphite (3.35 Å) there are two bond lengths that are substantially shorter: $d(C11-C22)=3.27$ Å and $d(C31-C32)=3.12$ Å.

FIG. 1 Characteristics of molecular C_{76} . *a*, HPLC traces of molecular carbon samples (1) after column-chromatography, with C_{76} , C_{78} and C_{84} peaks (in order of increasing retention time) prominent, and (2) after preparative HPLC separation, with only the C_{76} peak remaining (see text for details). *b*, A mass spectrum of C_{76} after the final stage of purification. The plot is a positive-ion laser-desorption (4.0 eV ; 0.1 mJ cm^{-2}) time-of-flight mass spectrum which shows, in addition to the dominant C_{76} peak and its normal mass-spectrometric fragment C_{74} , minor impurity peaks C_{78} and C_{84} at the 1–2% level, and a small C_{60} peak remaining as contaminant from an earlier calibration run. Deep-ultraviolet photoionization of laser-desorbed neutrals yields a similar spectrum. *c*, Optical spectrum of C_{76} (in liquid dichloromethane solvent at ambient temperature) across the ultraviolet (UV), visible (VIS) and near-infrared (NIR) spectral range. The inset shows an amplified view of the spectrum in the green to near-infrared region, emphasizing the absorption onset near 1.4 eV . Absolute extinction coefficients are as follows (sh stands for shoulder):

A	760 nm	[1.63 eV]	2,620 $\text{l mol}^{-1}\text{ cm}^{-1}$
B	715	[1.73]	3,080
C	572	[2.17]	2,600
D	524 (sh)	[2.37]	3,800
E	452 (sh)	[2.74]	10,840
F	408	[3.04]	16,280
G	356 (sh)	[3.48]	21,680
H	331	[3.75]	28,150
I	290	[4.28]	54,880
J	242	[5.12]	107,850

d, Infrared absorption spectrum of C_{76} in CS_2 solution recorded in a NaCl cell. Bands below 500 cm^{-1} and above $1,450\text{ cm}^{-1}$ are obscured by absorption by the window and solvent, respectively. The strongest band (near 700 cm^{-1}) is broadened and truncated by saturation.



the only substantial impurity. (3) The final step is a preparative high-pressure liquid chromatogram (HPLC) run on fraction II, performed on a Vydac 201-TP-510 column (25 cm, 1 cm internal diameter) with a 70:30 CH_2Cl_2 : CH_3CN eluant mixture (J. C. Fetzer and E. J. Gallegos, manuscript in preparation). The isolated, stable microcrystalline material was shown by mass spectrometry and HPLC profiles to be ~99% clean of all other carbon molecules.

Routine characterization is demonstrated in Fig. 1, which shows HPLC traces of the mixture and the pure material, together with a mass spectrum of the latter and the optical absorption spectra of C_{76} in the infrared region ($400\text{--}1,500\text{ cm}^{-1}$)

and the near-infrared/visible/ultraviolet region. An unusual feature is the near-infrared absorption, which has an onset near 890 nm (1.39 eV) and very large peak intensities (ϵ_{max} near $3,000\text{ l mol}^{-1}\text{ cm}^{-1}$) considering the cubic dependence of absorption on frequency. Both the solution phase and the crystalline form of the material are yellow-green when optically thin.

We have obtained structural information from ^{13}C NMR spectra (Fig. 2), taken in CS_2 solvent, in which C_{76} has a solubility greater than 1 mg ml^{-1} at room temperature. In contrast to our previous report, we obtain an interpretable spectrum after only 1,000 sweeps (4 s sweep time with $\text{Cr}(\text{acac})_3$ as relaxant) because of the high solubility, and the spectrum shown

was obtained after 38,000 scans. The spectrum consists of a series of 19 distinct lines of near-equal intensity, as confirmed by repeated scans, spanning the chemical shift region 130–150 p.p.m. Table 1 lists the line positions and their integrated intensities. An expanded view of the spectrum shows no other resonances in the ^{13}C NMR range, confirming high sample purity. The lines neatly span the same region as the five-line spectrum of C_{70} (refs 8, 10), and the centres of gravity of the three molecules are close: 143.2, 145.0 and 142.7 for C_{60} , C_{70} and C_{76} respectively. These facts point strongly to a fullerene structure.

The 19 lines of equal intensity evidently correspond to 19 distinct environments occupied each by four symmetry-equivalent carbon atoms. This strongly restricts the choice of structure. It implies a point-group consisting of four, and only four, elements (C_4 , S_4 , D_2 , C_{2v} , and C_{2h}), with none of the atoms lying on a symmetry element. Fullerene structures, consisting only of pentagonal and hexagonal rings, are inconsistent with C_4 , and also with C_{2v} when the atoms are excluded from the symmetry axis and mirror planes. Structures of C_{2h} symmetry require a belt of $4m$ hexagons around the mirror plane, and no plausible structure of this type exists. Here we use the strict definition of 'fullerene': a closed, hollow network of 12 pentagonal and m hexagonal rings for a C_{20+2m} molecule. Extensive experimentation with models has led to one plausible structure, illustrated in Fig. 3, which we believe to be unique. In particular, this structure has four groups of 'pyrene' sites—intersection of three six-membered rings—accounting for the four lines at chemical shifts well below 140 p.p.m. in perfect analogy to the description¹⁵ of C_{70} . We now describe the structure in greater detail.

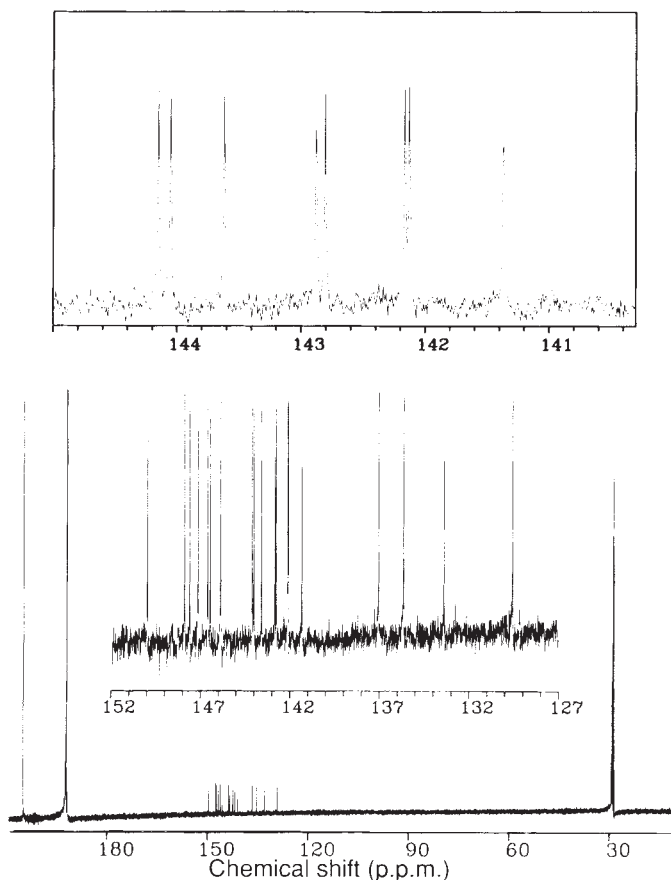


FIG. 2 ^{13}C -NMR spectra of 1.5 mg C_{76} in CS_2 , taken at 125.6 MHz, with acetone as an internal lock. (The acetone peaks are near 206 and 40 p.p.m., and the solvent peak is near 190 p.p.m.) The inset is an expanded view of the fullerene region. Closely spaced lines in the region 145.0–140.3 p.p.m. are expanded in the box above.

The molecule $D_{2h}\text{-C}_{76}$ has a fullerene structure; that is, it consists of 28 six-membered rings (6MR) and 12 five-membered rings (5MR), and it also obeys the pentagon isolation rule¹ by maximizing the separation of pentagons. Its relation to the C_{60} structure (in which each 5MR is surrounded by six 6MRs, and each 6MR is surrounded by alternating 5MRs and 6MRs) is most easily visualized through the following construction, illustrated in Fig. 3b. Take as each of two polar caps the pyracylene unit (the base unit of C_{60}), and surround them with alternating 5MRs and 6MRs to obtain the bold region of structure I. These last 5MRs in turn each require two additional hexagons to satisfy the pentagon-isolation rule, completing I. The resulting structures act as caps in C_{76} , and are each rather large fragments of C_{60} (ten 6MRs and four 5MRs). The crucial step (structure II) is the assembly of these caps at the edges of the last-mentioned 6MRs (9–12 and their equivalents); this requires that they be staggered by 20° about the long symmetry axis (a). The residual space around the perimeter is composed of two 6MRs and two 5MRs on each side.

A second, holistic view is gained by examining the connectivity of the rings in the assembled structure. As shown in Fig. 3d, it can be decomposed into a double-helix structure of edge-sharing pentagons and hexagons. The two strands are tied at the ends of the long axis by edge-sharing hexagons on the pyracylene bridges mentioned above. This view emphasizes the intrinsic helicity of the structure, which has no chiral centre.

A local, chemical view of the structure is obtained by considering the atomic environments (see Fig. 3b and c) of which there are three kinds. The most distinctive ('pyrene'-like) are the carbon atoms lying at the intersection of three hexagons, like those in the centre of a pyrene molecule. On each of the flattest faces (looking down the c -axis) there is a chain of six such sites snaking about a helix and crossing the axis, as shown by the shaded sites in Fig. 3c, structure C. On each of the most curved faces (looking down the b axis; Fig. 3c, structure B), there is a bonded pair of such atoms crossing the axis. These combine to give four groups of four atoms, and are assigned to the upfield (most shielded) peaks in the ^{13}C NMR spectrum. The second group encompasses the 'pyracylene' sites, labelled a to i in Fig. 3b, which are neatly grouped on the caps of the structure, each of which has six 5MRs and giving rise to nine pyracylene bridges with two sites each. All sites in C_{60} are like this, and the 40 sites in C_{70} having the least shielding are also like this. By analogy,

TABLE 1 Line positions in the ^{13}C NMR spectrum of C_{76} compared with C_{60} and C_{70} .

C_{76} δ (p.p.m.)	intensity	Type	C_{60}	C_{70}
150.03	(3.6)	[pc]*		150.8 (10) [pc]
147.96	(4.3)	["]		148.3 (20) ["]
147.66	(4.1)	["]		147.8 (10) ["]
147.19	(3.8)	["]		
146.65	(4.1)	["]		
146.50	(3.8)	["]		
145.92	(4.0)	["]		
144.14	(4.0)	["]		144.4 (20) [cor]
144.05	(4.1)	["]		
143.61	(4.0)	[cor]†		
142.86	(3.6)	["]	143.2 (60) [pc]	
142.79	(4.1)	["]		
142.15	(4.1)	["]		
142.11	(3.5)	["]		
141.35	(4.0)	["]		
137.06	(4.4)	[pyrene]		
135.67	(4.4)	["]		
133.40	(3.8)	["]		
129.56	(4.2)	["]		130.8 (10) [pyrene]

* Pyracylene.

† Corannulene.

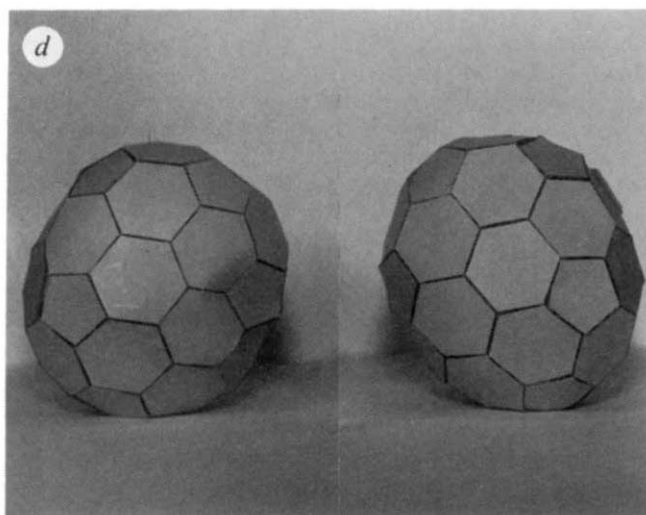
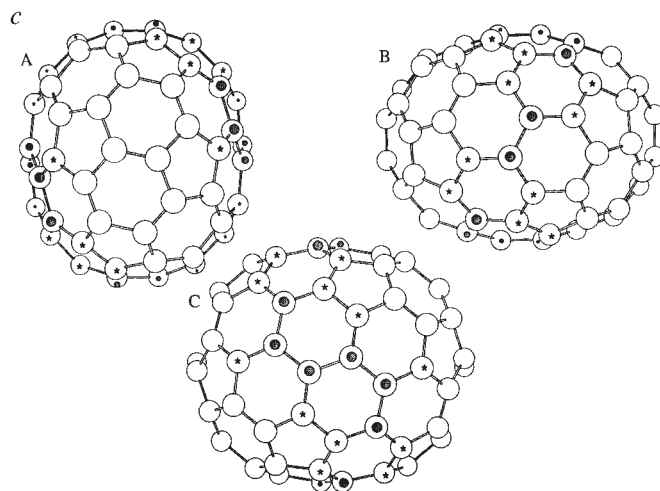
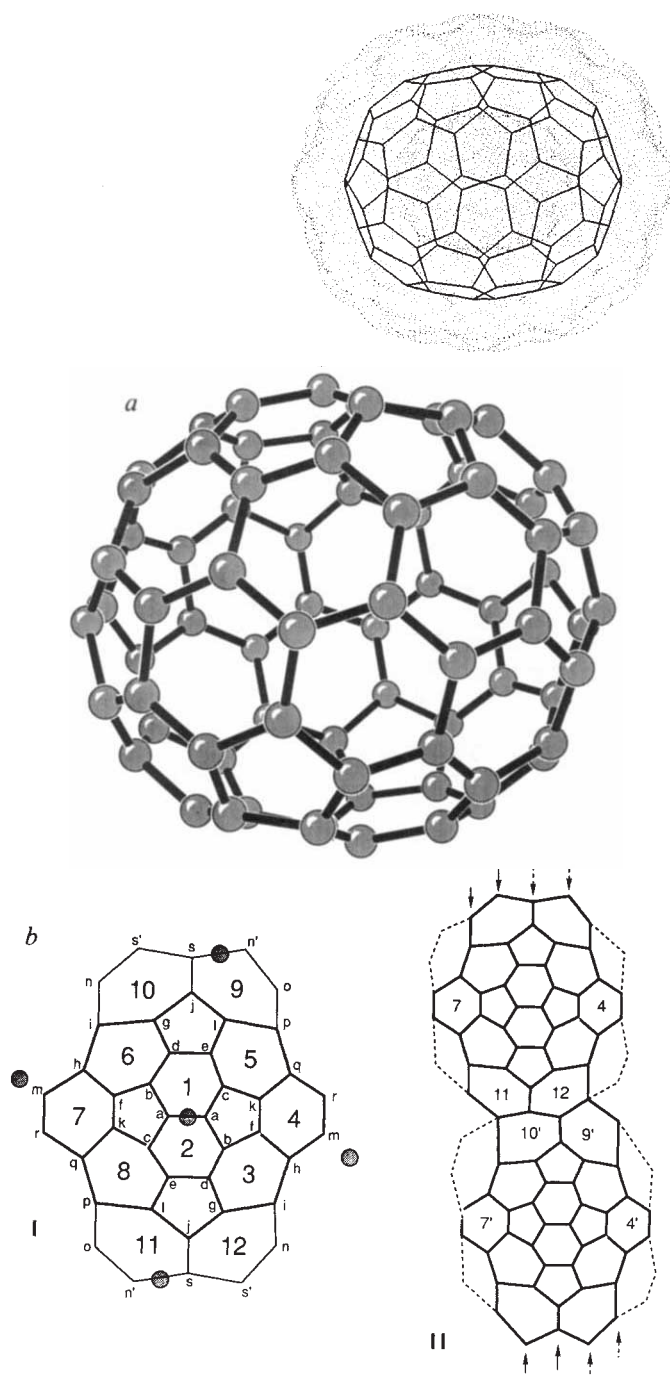


FIG. 3 Illustrations of the structure of C_{76} . *a*, Optimized structure of D_2 - C_{76} , with the long (*a*) axis horizontal, and looking down the short (*c*) axis. Inset: photograph of the simulated structure, with dot-cloud representing the interior and exterior van der Waals surfaces. *b*, Construction of D_2 - C_{76} from the pyracylene caps (bold area of structure I). The labelling of atoms (*a*)–(*s*) corresponds to the discussion in the text. *c*, Views along each of the three symmetry axes of D_2 - C_{76} . The shaded atoms are pyrene-type sites (*p*, *q*, *r*, *s* in *b*), to which the downfield peaks of the ^{13}C -NMR are assigned. The starred atoms are corrannulene sites (*j*–*o*), and the remainder are pyracylene sites (*a*–*i*). *d*, Photograph of paper models, showing the left- and right-handed forms.

we propose that the nine resonances above 144 p.p.m. correspond to these sites. The third kind of site, 'corrannulene', also lies at the intersection of one 5MR and two 6MR, but is not a pyracylene bridge (that is, it is not connected to another 5MR). There are 24 of these (six types labelled *j* to *o*) forming a disconnected boundary around the six caps, and we propose that these sites correspond to the six closely spaced resonances in the 141.3–143.6 p.p.m. region.

Thus the NMR spectrum can be assigned in detail to the proposed structure. Two-dimensional NMR could be used to establish this connectivity, as in the case of C_{70} (ref. 15).

We have examined the structure theoretically in several ways. First, we have performed Hückel molecular orbital calculations at the naïve level (a common β for all bonds), which reveal a small but significant HOMO–LUMO gap (0.344 β , or less than half of the 0.757 β found for C_{60} (ref. 17)) and a large delocalization energy (0.5551 β per atom, as compared with 0.5527 β for

C_{60} and the limiting value of 0.5743 for graphite). These values are in perfect agreement with those reported separately by Manolopoulos¹⁴. The HOMO–LUMO transition is allowed $b_1 \rightarrow a$. A reasonably optimized structure has been found through molecular mechanics calculations, and used as the initial structure in semi-empirical molecular orbital calculations (AM1-Gaussian 90)^{18–20}, within which the structure was further optimized. The important results are as follows.

(1) The D_2 symmetry is preserved with unconstrained optimization (Fig. 3). The bond lengths range from 1.37 to 1.47 Å, and show an alternation similar to that described for C_{60} . The dimensions along the three symmetry axes (*a*–*c*) are $D_a = 8.79$ Å, $D_b = 7.64$ Å, $D_c = 6.68$ Å, as compared with 7.0 Å for C_{60} . The density should correspondingly be 25–30% lower than that of C_{60} . (2) The estimated standard heat of formation (relative to graphite) is $\Delta H_f = +14.75$ kcal mol^{−1} atom^{−1}, somewhat lower (more stable) than the estimate for C_{60} (+16.21 kcal

$\text{mol}^{-1} \text{atom}^{-1}$) at the same level of theory. (3) The estimate for the HOMO–LUMO gap is large, about 80% of that calculated for C_{60} (5.4 compared with 6.7 eV, although these greatly overestimate the optical transition energies), and the locations of the HOMO (–8.9 eV) and LUMO (–3.5 eV) indicate that it should be both a better donor and a better acceptor than C_{60} (corresponding locations –9.6 eV and –2.9 eV).

The electronic spectrum in the near-infrared is consistent with this theoretical description. The onset at 1.39 eV and first maximum or shoulder near 1.5 eV can be compared to the values for C_{60} (2.0 and 2.2 eV), and the transitions are strong enough to be fully allowed. Magnetic properties would also be of interest for comparison with those of C_{60} and C_{70} and when considering the aromaticity of fullerenes^{21–23}. Consistent with the relatively low symmetry of the structure, the infrared spectrum provides a very complex fingerprint, with irregular structure dominated by strong bands at 660, 747, 1,035, 1,127 and 1,434 cm^{-1} . This spectrum should provide a sensitive test for theoretical calculations based on model potentials. We anticipate that these and higher-level calculations could be used to predict and explain interesting electronic and optical properties of the molecular and pure-solid materials.

We have shown that the third molecular form of carbon belongs to the fullerene family, as anticipated, but that it has highly unusual symmetry properties, as expressed in its point group (D_2) and more specifically in its chirality, which is based on helicity in the absence of a single chiral centre. These properties are interesting, unprecedented and largely unexpected. Recently, Fowler¹⁶ has put forward a structural proposal for C_{84} , another abundant higher fullerene, having the same symmetry. Close analysis of this D_2 – C_{84} structure (ref. 14, and P. Labastie, H.-P. Cheng and R.L.W., manuscript in preparation) reveals that it has the same kind of helicity as D_2 – C_{76} . It remains to be seen whether this is the natural form of C_{84} . There is also the interesting question of why these helical forms might be preferred to the cylindrical or cubic forms discussed earlier¹³.

The chiral nature of D_2 – C_{76} implies that the two enantiomeric forms could be separated to yield elemental optically active materials. The optical properties, such as coefficients for optical rotation, may have interesting applications.

Much remains to be done to characterize this material, its chemical, electronic and magnetic properties, its detailed structure, its solid-state properties and so on. At first inspection it appears to be one of the most fascinating molecular architectures ever seen in nature. We expect that with the worldwide attention given to scaling up production of C_{60} , gram-size quantities of C_{76} will be available in the next few years. □

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Decrease in anthropogenic lead, cadmium and zinc in Greenland snows since the late 1960s

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MORE than twenty years ago, Patterson and co-workers¹ showed that evidence of lead concentrations in Greenland ice and snow had increased about 200-fold since ancient times. From their results, they concluded that more than 99% of this highly toxic metal in the global troposphere of the Northern Hemisphere originated from human activities in the mid 1960s—mainly from the use of alkyl-lead petrol. At least in part because of this evidence, the United States and other countries limited the use of lead additives in petrol from about 1970. Here we report that, as a result of these policy initiatives, lead concentrations in Greenland snow have decreased by a factor of 7.5 over the past twenty years. We also show that over the same time period, cadmium and zinc concentrations have decreased by a factor of 2.5.

As many previous investigations of heavy metals in Greenland snows have been hampered by contamination problems (see recent reviews in refs 2–5), we first describe our sampling and analytical procedure in some detail. Samples were taken on 19–21 July 1989 in central Greenland, near Summit (72° 35' N, 37° 38' W, elevation 3,230 m, mean annual snow accumulation rate 21.5 $\text{g cm}^{-2} \text{yr}^{-1}$) as part of the European Eurocore program. The samples covered a continuous sequence of 22 years (1967–1989). They consisted of a snow core 10.5 cm in diameter and 10.7 m deep, which was drilled only for our heavy metal measurements, and of a few additional shallow samples. To minimize local contamination, the sampling site was selected several kilometres north of the Eurocore camp, in a clean area to which access was limited. The core was hand-drilled by operators wearing full clean-room clothing and shoulder-length polyethylene gloves, using a specially designed SIPRE-type all-plastic mechanical auger made of polycarbonate. The cutting blades, the extension rods and the T-shaped arm used to rotate the auger were also made of polycarbonate. Before being sent to Greenland, the auger, extension rods and all other items (also made of plastic) used during drilling operations had been extensively cleaned in our clean laboratory⁶ by successive immersions in a series of 25% and 0.1% HNO_3 baths⁶, the last two washes being ultrapure 0.1% HNO_3 (ref. 7; from US National Institute of Science and Technology) diluted in ultrapure water⁶. The core sections (length ~50 cm) were directly transferred by gravity into specially designed conventional polyethylene tube with threaded caps, which had also been extensively cleaned in the clean laboratory and were packed in multiple sealed acid-cleaned polyethylene bags. All samples were brought back frozen to France.

Despite the exceptional cleanliness of the field collection procedure, the possibility remained that significant, although probably very limited, heavy metal contamination might be present on the outside of the snow core. Each core section investigated was therefore subsampled through freshly prepared untouched surfaces, inside a laminar flow clean bench located

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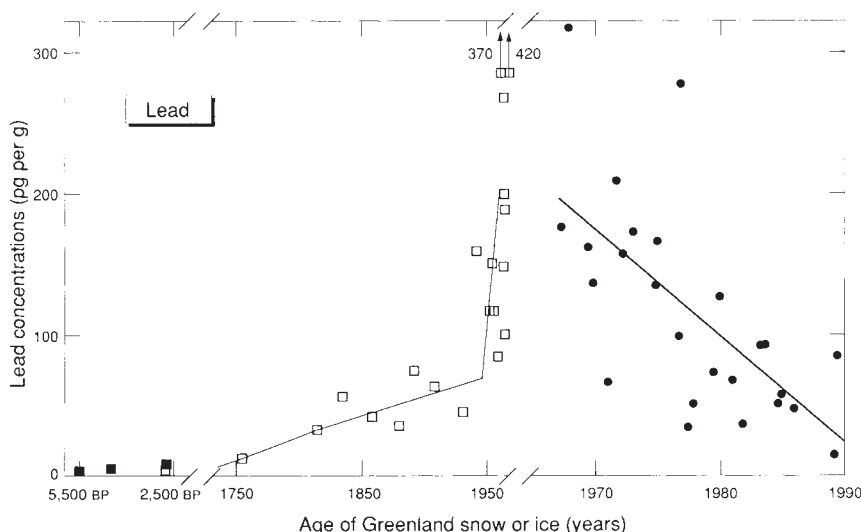


FIG. 1 Changes in Pb concentrations in Greenland ice and snow from 5,500 BP to present. Full dots (right): data from 1967 to 1989, from Summit, this work. Open squares (centre): 1753 to 1965, from north-west Greenland¹ (the corresponding curve shown is the original one given in ref. 1). Full and open squares (left): 5,500 BP to 2,700 BP data from Camp Tuto and Camp Century respectively^{1,12}. Although these data were obtained at widely dispersed locations, it seems reasonable to put them together as the concentrations of major impurities such as Na, Al and sulphates are similar at these sites. After a great increase (~200-fold) from several thousand years ago to the mid 1960s, Pb concentrations have decreased rapidly (by a factor of ~7.5) during the past twenty years, mainly as a consequence of the fall in use of lead additives in gasoline.

inside a cold room, following a procedure similar to that described in ref. 8. From each section of initial length ~50 cm, we could first obtain two ~20-cm-long core sections, with fresh untouched surfaces at both extremities, by breaking the original core section with a sharp-edged polyethylene splitting wedge (used only to initiate the crack) and a polyethylene hammer. The central part of each of these two sections is then extracted by hammering a home-made cylindrical polyethylene beaker (5 cm diameter, 20 cm long) into the centre of one of these untouched surfaces parallel to the axis of the core axis. Additional subsamples are also taken parallel to the axis at increasing distances from the centre of each section using narrower (1 cm diameter, 20 cm long) conventional polyethylene beakers. All items used during subsampling were extensively cleaned following the procedure described in detail in ref. 6.

Twenty-five ~20-cm-long sections were subsampled, representing about half of the total length of the core. Each subsample was analysed separately for Pb, Cd and Cu by graphite furnace atomic absorption spectrometry (GFAAS) after preconcentration by non-boiling evaporation⁹ and for Zn, Al and Na by GFAAS without preconcentration. Some were also analysed for Cd by direct ultrasensitive laser-excited atomic fluorescence spectrometry (LEAFS)^{10,11}, giving results which agree well with those from GFAAS (Table 1). All results were carefully corrected for the blank contributions originating from the successive steps of the analytical procedure (these procedural blanks from the subsampling stage onwards always represent less than 10% of metals intrinsic to the samples). Precision of the data was estimated to be ~10–20%.

We investigated the variation of the measured concentrations from the outside to the centre of each core section in detail to determine whether the concentrations measured in the centre represented the original concentrations in the snow. In most cases, concentrations were fairly constant across the core,

without any significant contamination on the outside. Significant although rather limited contamination, especially of Cd and Cu, was present in the outside layer of a few core sections, but the concentrations always reached a good plateau in the central parts of these core sections.

The measured variations in concentration of the heavy metals at Summit as a function of the age of the snow are shown in Fig. 1 for Pb (also shown are the Greenland Pb data for the period 5,500 BP to 1965, from refs 1, 12) and Fig. 2 for Cd, Zn and Cu. The dating is based on the seasonal variations of methanesulphonic acid (M. Legrand, personal communication); its precision is better than 0.5 yr. These profiles form comprehensive records of heavy metals in Greenland snows from the mid 1960s to present. Other published data on heavy metals for this period come from only a few samples covering very limited periods (a year at the most) collected at widely dispersed locations^{13–16}.

The natural contribution to the heavy metal from rock and soil dust in snow at Summit can be calculated from the Al concentrations (ranging from 1.4 to 17 ng per g) measured in our samples, using the mean metal/Al ratios in rock or soil^{17,18}. As illustrated in Table 2, this contribution is very small (from ~2% down to ~0.2%) for Pb and Cd. For Zn, it is higher, but is always $\leq 20\%$. In contrast, the contribution for Cu is significant throughout the samples: for ~1/3 of the samples, it is close to the measured concentrations; for the others, it usually represents more than 50%, which indicates that Cu and Al values are significantly correlated. After correction for contribution from rock and soil dust, we used our Na concentration values (ranging from 1.7 to 19 ng per g) to estimate the natural heavy metal contribution from sea-salt spray from the mean metal/Na ratios in bulk sea water¹⁹, combined with the few published enrichment factors²⁰ relative to bulk sea water for these metals in sea-derived aerosols. This contribution is found to be negligible. The other possible natural contributions are from volcanoes, forest fires and continental or marine biogenic sources²¹. They cannot be evaluated from our data. But simple considerations using the best published estimates of the natural fluxes of metals from these sources to the global atmosphere²¹ indicate that their contributions are probably negligible for Pb, Cd and Zn, but possibly significant for Cu. From this discussion, it appears that Pb, Cd and, to a lesser extent, Zn in snow from 1967–1989 at Summit are almost entirely derived from anthropogenic sources, whereas a large fraction of Cu is natural. Similar conclusions have been reported previously¹⁵ for these four metals in snow representing one year's accumulation (1983–84) from near Dye 3 in south Greenland.

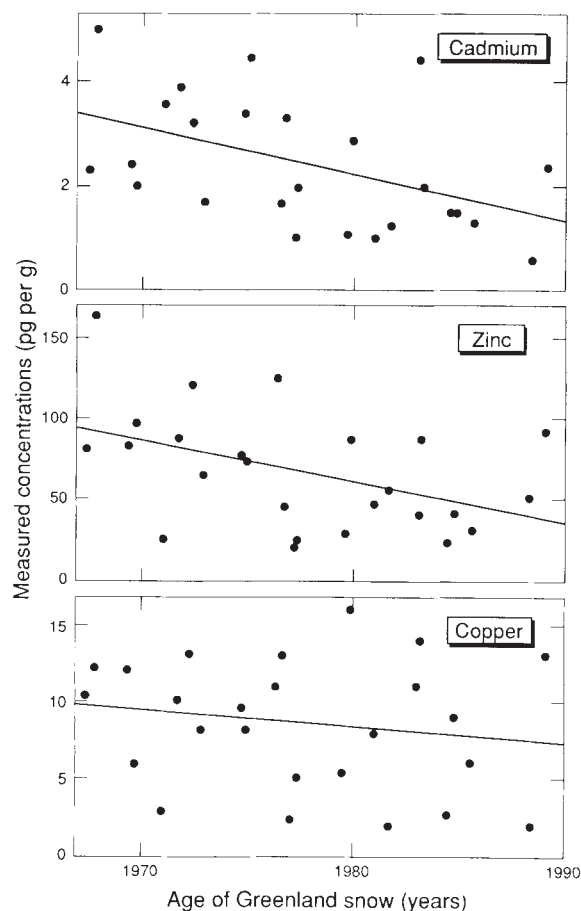
As shown in Figs 1 and 2, the concentrations of heavy metals at Summit from 1967 to 1989 vary widely. This is probably

TABLE 1 Cadmium in snow cores from Summit, Central Greenland

Depth (m)	Measured Cd concentrations (pg per g)	
	GFAAS after preconcentration	LEAFS
1.90–2.03	1.3 ± 0.3	1.4 ± 0.8
3.32–3.47	2.0 ± 0.4	2.2 ± 1.2
6.40–6.52	3.3 ± 0.7	2.9 ± 1.6
8.77–8.87	3.9 ± 0.8	3.7 ± 2.1

Comparative determination of Cd in the central part of four sections of a 10.7-m snow core by graphite furnace atomic absorption spectrometry (after preconcentration by non-boiling evaporation), and by direct laser-excited atomic fluorescence spectrometry.

FIG. 2 Changes in Cd, Zn and Cu concentrations in snow at Summit, central Greenland, from 1967 to present. The least-square fits shown for each metal indicate that since 1967, Cd and Zn concentrations have decreased by a factor of ~ 2.5 , probably as a consequence of the abatement policies for industrial emissions. Concentrations of Cu, which is mainly derived from natural sources, appear on the other hand to have remained fairly stable.



mainly because each data point represents a rather short and variable period (ranging from ~ 3 to 6 months) so that our data are certainly influenced by short-term changes such as seasonal effects^{1,15}. The least-square fits in Figs 1 and 2, however, allow us to establish the general time trends for each metal. From 1967 to 1989, Pb concentrations are found to have strongly decreased (by a factor of ~ 7.5), while Cd and Zn have decreased less strongly (by a factor of ~ 2.5). The corresponding gradients (with confidence intervals at the 0.80 confidence level) are -7.95 ± 1.86 pg per g per year for Pb, -0.090 ± 0.035 pg per g per year for Cd and -2.58 ± 1.08 pg per g per year for Zn. On the other hand, the very slight decrease observed for Cu (by a factor of ~ 1.3 , with a gradient of -0.23 ± 0.16 pg per g per year) appears to be barely significant, which is consistent with the fact that Cu is probably mainly natural. This demonstrates that the concentrations in Cd and Zn in Greenland snow have decreased during the past two decades. For Pb, such a decrease was tentatively suggested by Wolff and Peel¹⁵ to explain the low

Pb concentrations measured near Dye 3 for snow laid down in 1983–84.

The observed decreases of Pb, Cd and Zn concentrations in central Greenland snows indicate that the pollution of the global troposphere of the Northern Hemisphere has significantly decreased during 1967–1989 for these three metals, and that this decrease was especially pronounced for Pb. The decrease for Pb follows the increase by a factor of ~ 200 from 5,500 BP to the mid 1960s (Fig. 1) which was reported by C. Patterson and co-workers^{1,12}. For Cd and Zn, the decrease probably also follows a previous increase, but this will need to be confirmed by obtaining reliable time series for these two metals through the industrial period (reliable Greenland time series for heavy metals other than Pb are not available at present).

There has been a rapid fall in the use of Pb additives in gasolines during the past twenty years in the Northern Hemisphere, especially in North America and western Europe (refs 22–24; and J. O. Nriagu and J. M. Pacyna, personal communications). In the United States, for instance, the consumption of Pb additives fell by more than 90% between the late 1960s and present. Clearly, our data confirm that this fall has resulted in a very large decrease in the large-scale pollution of the troposphere of the Northern Hemisphere by this highly toxic metal.

For Cd and Zn, the situation is rather different. In contrast to Pb, for which only a small fraction of the anthropogenic emissions to the atmosphere originated from causes other than gasoline additives in the 1960s (ref. 25), anthropogenic Cd and Zn originated mainly from industrial processes such as the combustion of fossil fuels, nonferrous metal production, steel and iron manufacturing and incineration of refuse²⁵. Our data indicate that the increasing efforts made in various Northern Hemisphere countries to reduce industrial emissions of Cd and Zn, and of other metals and pollutants (see, for example, refs

TABLE 2 Crustal enrichment factors in snow cores from Summit, central Greenland

Depth (m)	Year	Crustal enrichment factor*			
		Pb	Cd	Zn	Cu
0.05–0.25	1989	32	58	6.3	1.2
10.49–10.65	1967	230	186	19	3.0

* (Defined as metal/Al ratio in sample divided by metal/Al ratio in mean crustal material¹⁷) in snow from 0.05–0.25 m and 10.49–10.65 m (top and bottom sections of the snow core, respectively). Enrichment factors close to unity indicate that rock and soil dust makes a large contribution to the measured metal concentrations. High enrichments indicate that rock and soil dust contribution is negligible.

26, 27), have now resulted in a significant decrease in large-scale tropospheric pollution by these two metals. □

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Radiocarbon test of earthquake magnitude at the Cascadia subduction zone

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THE Cascadia subduction zone, which extends along the northern Pacific coast of North America, might produce earthquakes of magnitude 8 or 9 ('great' earthquakes) even though it has not done so during the past 200 years of European observation¹⁻⁷. Much of the evidence for past Cascadia earthquakes comes from former meadows and forests that became tidal mudflats owing to abrupt tectonic subsidence in the past 5,000 years^{2,3,6,7}. If due to a great earthquake, such subsidence should have extended along more than 100 km of the coast². Here we investigate the extent of coastal subsidence that might have been caused by a single earthquake, through high-precision radiocarbon dating of coastal trees that abruptly subsided into the intertidal zone. The ages leave the great-earthquake hypothesis intact by limiting to a few decades the discordance, if any, in the most recent subsidence of two areas 55 km apart along the Washington coast. This subsidence probably occurred about 300 years ago.

One clue about the magnitude of a prehistoric earthquake is the extent of sudden tectonic deformation judged coeval by radiocarbon dating. The length of the region deformed tectoni-

cally during an earthquake increases with the length of fault rupture^{8,9}, and fault-rupture area largely controls the magnitude of the earthquake^{10,11}. Radiocarbon dating can lack the precision to show whether regional deformation was accomplished during a single great earthquake or was 'mosaicked' from a brief series of lesser shocks^{9,12}. But dating can put upper bounds on magnitude by showing the maximum length of deformation attributable to a single earthquake, and it can also indicate lower bounds for magnitude if serial earthquakes are likely to differ in age by an amount that the dating can resolve.

Conventional radiocarbon dating has not revealed whether the youngest sudden subsidence in southern coastal Washington was coeval. Conventional ages have shown only that this subsidence postdates AD 1400, and that it comprised anything from a single event to a series of events distributed through several centuries⁷. This low resolution reflects three problems. First, some of the ages were measured on materials, including peat and sticks, that may predate or postdate the subsidence by tens or hundreds of calendar years. Second, the quoted errors for most of the ages are 50-100 ¹⁴C years, and unknown analytical errors may make standard deviations still larger¹³. Third, even allowing for only the quoted errors many of the individual ages could correspond to one of several calendar dates that range from the late 1600s to 1955, an ambiguity inherent in most conventional radiocarbon ages less than 300 ¹⁴C years before present (BP)^{14,15}.

We tested coeval subsidence more rigorously by subjecting nine stumps of Sitka spruce (*Picea sitchensis*) to radiocarbon dating with quoted errors of 10-20 ¹⁴C years, a much higher precision than is commonly available. All nine stumps are rooted in the uppermost forest soil buried by Holocene tidal mud, six of them at Willapa Bay, the other three 55 km to the north along the Copalis River (Figs 1b, 2). The stumps retain unweathered rings that adjoin bark. Evidence reported elsewhere^{2,6,7} shows that such outer rings should have formed within a few years of earthquake-induced subsidence; the evidence includes incomplete or wide rings that suggest normal growth until a tear or two before death (Fig. 2, I and W)⁶. We did not attempt to date the spruce by their ring-width patterns because only the spruce roots have escaped deep decay, and these lack long series of tabular rings. We did, however, use ring-pattern dates from more durable fossils as a basis for guessing which spruce-root rings

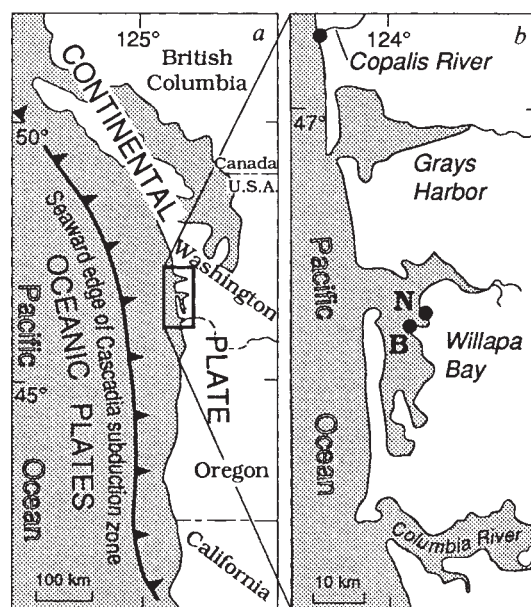
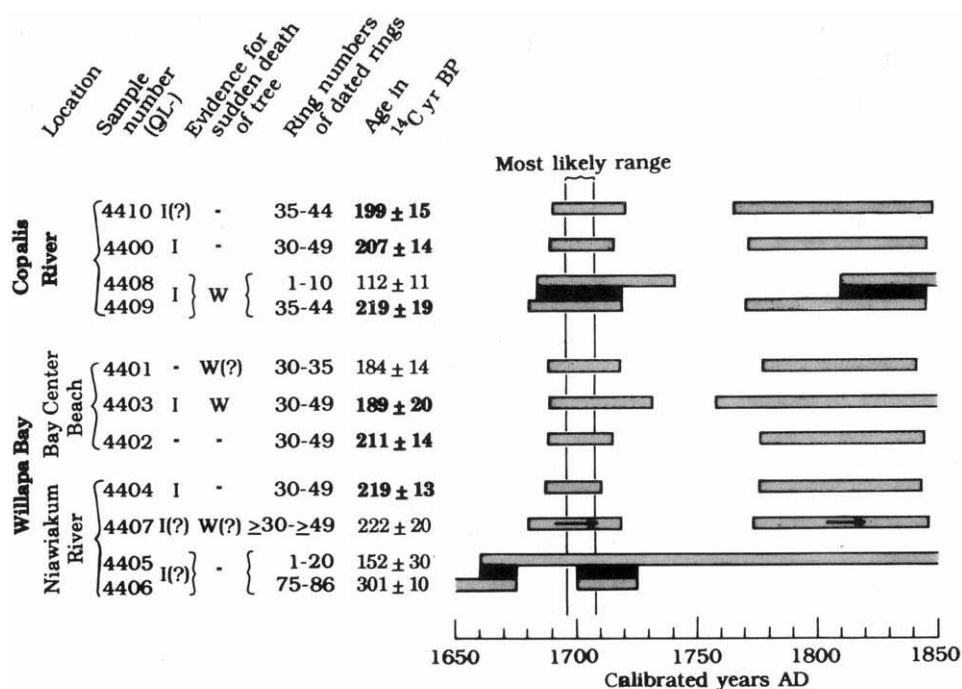


FIG. 1 Index maps. a, Cascadia subduction zone and vicinity. Arrows show direction of dip of boundary between subducting oceanic plates and over-riding continental plate. b, Southern coastal Washington and vicinity. Circles denote radiocarbon localities. B, Bay Center beach; N, Niwaiakum River.

FIG. 2 Radiocarbon ages of tree rings from spruce stumps and corresponding calibrated (approximately calendric) ages for tree death. On the left-hand side, I indicates that the ring adjoining bark is incomplete; W, widths of outer rings show little or no outward decrease. Each ring-number pair gives interval, in tree-ring years, between formation of dated rings and death of tree; ring 1 adjoins bark. 'Age in ^{14}C years BP' denotes conventional radiocarbon years before AD 1950; quoted error is standard deviation based on counting statistics of samples and standards only. The right-hand side shows the ranges of calibrated ages for tree death before AD 1850, at >95% confidence. Black bars emphasize overlap between tree-death ages from different ring blocks of same stumps (example in Fig. 4); open-ended bars extend beyond scale at bottom (complete bars shown in Figs 3 and 4); the two bars containing arrows denote limiting ages from a root in which the decayed periphery may lack rings from the final few years before tree death (QL-4407). 'Most likely range' gives the earliest group of tree-death ages corresponding to weighted mean of radiocarbon ages listed in bold face (209.3 $\pm$ 6.0 ^{14}C years BP).

METHODS. Slabs were cut from bark-bearing parts of spruce stumps at Copalis River estuary (47° 07.10' N, 124° 09.97' W), Bay Center beach (46° 37.82' N, 123° 57.42' W) and Niihau River (46° 36.68' N, 123° 53.60' W) (Fig. 1b). From each slab we removed 6–20 consecutive rings, the mass of which was roughly symmetrical about the ring with mean ring number. Most samples weighed ~85 g before chemical treatment. Preparation of cellulose for dating was analogous to

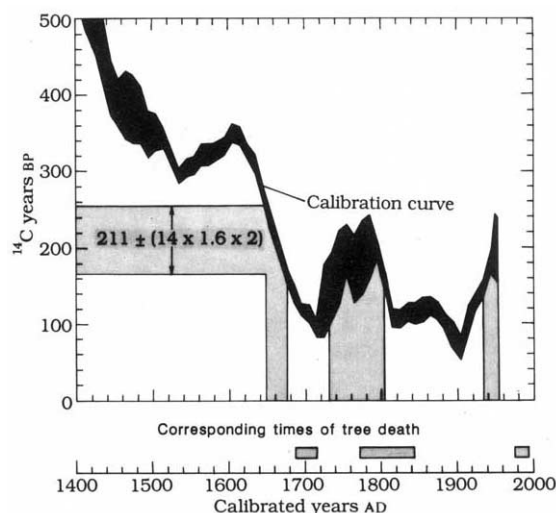


^{13}C sample preparation described previously¹⁸. The age for QL-4408 is the weighted mean of three values (103 $\pm$ 19, 106 $\pm$ 22 and 125 $\pm$ 18 ^{14}C years BP) from counts made at times distributed through period when other samples were counted. Figures 3 and 4 illustrate the procedure for estimating calibrated ages of tree death.

might yield unambiguous radiocarbon ages. These fossils are standing dead trunks (snags) of western red cedar (*Thuja plicata*) that protrude through tidal marshes in coastal southern Washington and are rooted, like the dated spruce stumps, in the uppermost buried soil^{6,7}. Preliminary matching of ring-width patterns shows that the red-cedar snags, which have lost outer rings to decay, died some time after the early 1680s at all four estuaries named in Fig. 1 (D.K.Y., unpublished data). On this basis, we sampled spruce-root rings that average 40 tree-ring years before tree death (Fig. 2) when aiming to exploit the unambiguous relation between high-precision radiocarbon ages near 250 ^{14}C years BP and calibrated (approximately calendric) ages near AD 1650 (refs 14, 15; Fig. 3).

The high-precision ages give no evidence against coeval subsidence. The ages best suited to falsify coevality come from the six stumps that yielded large samples of 10 or 20 rings averaging tree-ring 40 years before tree death (ages listed in bold face in Fig. 2). The weighted means of these ages differ between the Copalis River (207 ^{14}C years BP) and Willapa Bay (210 ^{14}C years BP) by less than one-third of the standard errors of these means (weighting by inverse square of quoted errors). Moreover, all these ages overlap with one another at one quoted error (13–20 ^{14}C years), which probably includes 0.6–0.8 standard deviation in the measurement of ^{14}C age¹⁵. This overlap implies tree death within the same few calendar decades if the trees died between 1680 and 1720, for in that case the bold-face ages

FIG. 3 Conversion of a radiocarbon age to ranges of calibrated (approximately calendric) ages for death of a spruce. The ring block is QL-4402 containing rings formed 30–49 years before the death. The conversion uses the calibration curve of Stuiver and Becker¹⁴, who measured ^{14}C ages of rings of known tree-ring ages; the width of the curve shown here represents two standard deviations in ^{14}C age. Error quoted for radiocarbon age (Fig. 2) is multiplied by 1.6 (ref. 15) and then doubled, so that the error plotted probably includes two standard deviations. Calibrated ages for tree death are younger than calibrated ages for sample by an amount equal to ring number of mean ring in sample. Ring 1 adjoins bark.



represent a time (middle or late 1600s) for which 20 ^{14}C years span less than a calendar decade¹⁴ (Figs 3, 4).

All the high-precision ages are consistent with tree-killing subsidence between 1680 and 1720 (Fig. 2). Nearly all these ages are also consistent with subsidence during two other intervals, 1770–1850 and 1950–1995 (example in Fig. 3). But three points favour 1680–1720: an age obtained from interior rings indicates that tree death was no later than 1730 (QL-4406, Fig. 4); an age from exterior rings contradicts tree death in the late 1700s (QL-4408, Fig. 2); and a botanical description¹⁶ of western red cedar as the only type of snag in Willapa Bay tidal marshes of 1853 implies tree death early enough for spruce to have decayed to buried stumps by the middle 1800s. If the dated spruce died between 1680 and 1720, and if the ages listed in bold face are coeval, then the weighted mean age for these ages (209 ± 6 ^{14}C years BP) puts the 95% confidence interval for tree death between 1695 and 1710 ('Most likely range', Fig. 2).

We conclude that two areas 55 km apart along the subduction-zone coast were last struck by sudden subsidence within the same few decades around AD 1700. This coincidence implies coordinated movement on a single fault rather than random slip on two unconnected faults. The only active fault common to both dated areas is the plate boundary that descends gently eastward beneath coastal Washington¹⁷ (Fig. 1a). Our initial dating covers too little coast to test for a plate-boundary rupture that would have been long enough for an earthquake of magnitude 8 or 9. But the dating fails to suggest a rupture shorter

than 55 km, and this failure strengthens the case for great earthquakes at the Cascadia subduction zone. □

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Unusual sea-floor fabric near the Bullard fracture zone imaged by GLORIA sidescan sonar

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THE tectonic fabric of the sea floor created at spreading ridges generally curves in the direction of ridge offset near transform faults, owing to the influence of shearing stress on transform faults. Here we present new GLORIA sidescan sonar images from the South American–Antarctic Ridge, showing fabric to the north of the Bullard transform with a reversed sense of curvature. This curvature, and that observed in an area of older sea floor near the eastern ridge–transform intersection, appears to result from post-emplacement deformation, caused by the transmission of shear stresses across transforms. The deformation was probably related to a major Early Miocene change in spreading direction, which would have imposed a component of compression on the transforms. Such a sense of curvature has not, to our knowledge, been observed elsewhere on the deep ocean floor, although apparently similar patterns have been reported in ophiolites.

During the first deployment of the IOS long-range sidescan sonar GLORIA in the Southern Ocean in 1989, two swaths of imagery were collected near the 550-km offset Bullard fracture zone (Fig. 1), which lies on the South American–Antarctic Ridge system (SAAR), midway between the southern South Sandwich trench and the Bouvet triple junction. The results illustrate the changes in geometry of the SAAR resulting from a change in spreading direction from 120°–300° to roughly east–west, which occurred shortly before the time of magnetic anomaly 6 (20 Myr BP). Previous work¹ indicated that the creation of long-offset transforms on the SAAR was a consequence of the change in spreading direction, and that several spreading ridge segments must have been extinguished as the new Bullard transform broke through pre-existing lithosphere.

Present-day motion between the South American and Antarctic plates (SAM–ANT) on the SAAR is slightly south of west

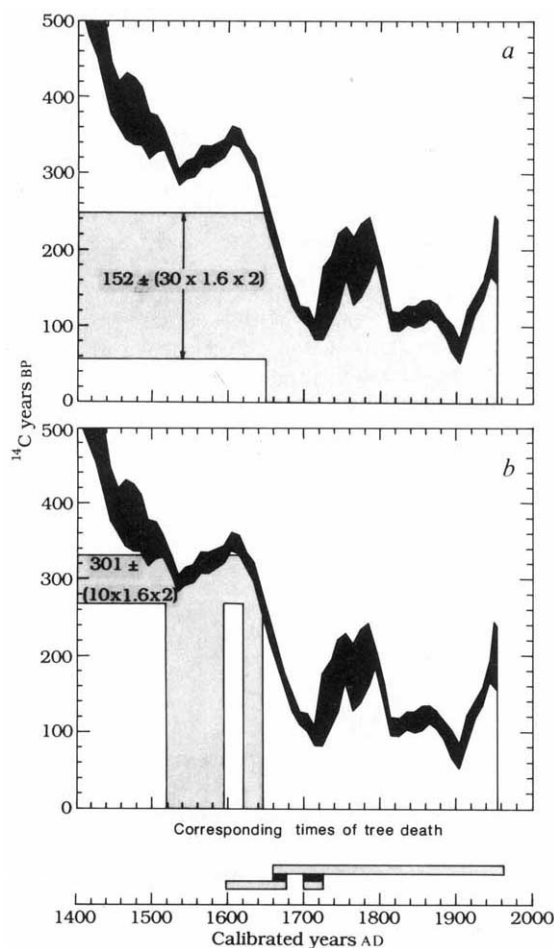


FIG. 4 Same as Fig. 3, for two different ring blocks from a single spruce at Willapa Bay. *a*, Rings formed 1–20 years before tree death (QL-4405). *b*, Rings formed 75–86 years before tree death (QL-4406). Black bars at bottom show tree-death ages consistent with both ^{14}C ages.

and slow, with full rates¹⁻⁴ of 18–22 km per Myr (Fig. 1). Typical of slow-spreading ridges, the SAAR is characterized by deep median valleys (up to 4,900 m), deeper transform valleys (up to 6,400 m) and rough topography^{2,3}. The active Bullard transform has a deep transform valley flanked to the south by a linear ridge shoaling to <3,000 m, with 'nodal basins'⁵ exceeding 5,000 m depth near the ridge-transform intersections (RTIs).

A digital GLORIA mosaic of seafloor adjacent to the Bullard transform, and a map of lineaments based on this imagery are shown in Fig. 2. The transform zone itself was not imaged, as our primary aim was to map flowlines of SAM-ANT relative motion. To the southeast of the transform, three fracture zones (FZs) can be seen curving to the southeast away from the transform zone. The westernmost FZ (A) forms the eastern edge of an area of typical sea-floor spreading fabric, the strike of which seems to remain orthogonal to the FZ as its trend changes. This fabric was probably created at the ridge that now joins the Bullard transform at the western RTI (Fig. 1). It seems that the ridge was able to adjust to the change in spreading direction by gradual rotation, perhaps by differential asymmetric spreading in the manner suggested by Menard and Atwater⁶ for the north-eastern Pacific. To the east of FZ A is a compartment of very low backscatter, suggesting older, more heavily sedimented crust, bounded in turn by a second FZ (B) which was truncated when the Bullard transform broke through.

East of FZ B, a second extinct spreading compartment is observed, with broad, bright reflectors which may represent spreading fabric created before anomaly 6. This compartment is separated by another truncated FZ (C) from a compartment in which bright, narrow NNW-SSE-oriented reflectors are seen to curve in an anticlockwise sense within ~20 km of the inactive eastern extension of the Bullard transform. Images of the area

to the east of 6° W (Fig. 2a) show that this curvature extends into crust adjacent to another truncated FZ (D), which trends away from the Bullard fracture zone at a shallow angle.

The active spreading ridge segment north of the Bullard FZ is imaged at ~7° W. Bright returns are seen from young volcanics and fault scarps close to the rift, even though they strike perpendicular to the ship's track, and further bright returns are obtained from the southern wall of the nodal basin near the RTI. To the west, the level of acoustic backscatter declines, reflecting the increase in sediment thickness with crustal age. At ~9° W, where the crustal age is estimated to be ~10 Myr, the fabric begins to rotate progressively in an anticlockwise sense, this broad curvature being maintained in the older sea floor to the west.

Curved ridge-crest fabric adjacent to fracture zones has been observed using long-range sidescan sonar imagery and/or swath bathymetry on principal spreading systems worldwide, at a variety of spreading rates and offsets (for example, refs 7, 8; Fig. 3a). It results from a gradual reorientation of ridge-parallel fault-scarps and volcanic constructs within ~5–20 km of the active transform and its extensions, so that they curve toward the direction of ridge offset, and is usually apparent in fabric on both sides of the ridge, as well as in the trend of the ridge itself^{8,9}. This is generally attributed to dyke injection and normal faulting in a stress field in which the direction of minimum compressive stress is rotated under the influence of shearing stresses associated with the transform¹⁰⁻¹³. The curvature seen in the imagery from near the Bullard transform, however, is in the opposite sense, that is, away from the direction of ridge offset, suggesting an anomalous stress field (Fig. 3b), and extends 30 km or more north of the transform.

Patterns of curved sea-floor fabric have been observed at other

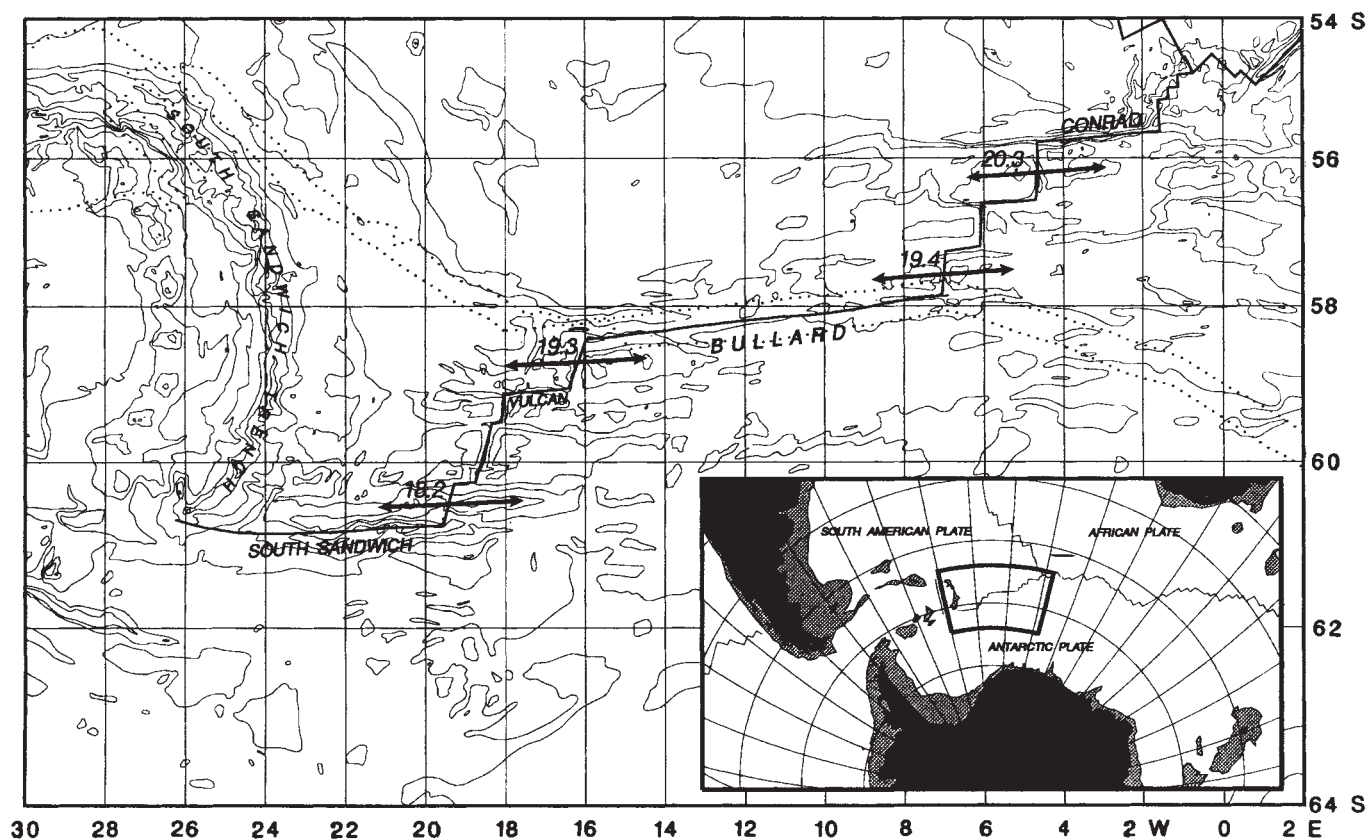
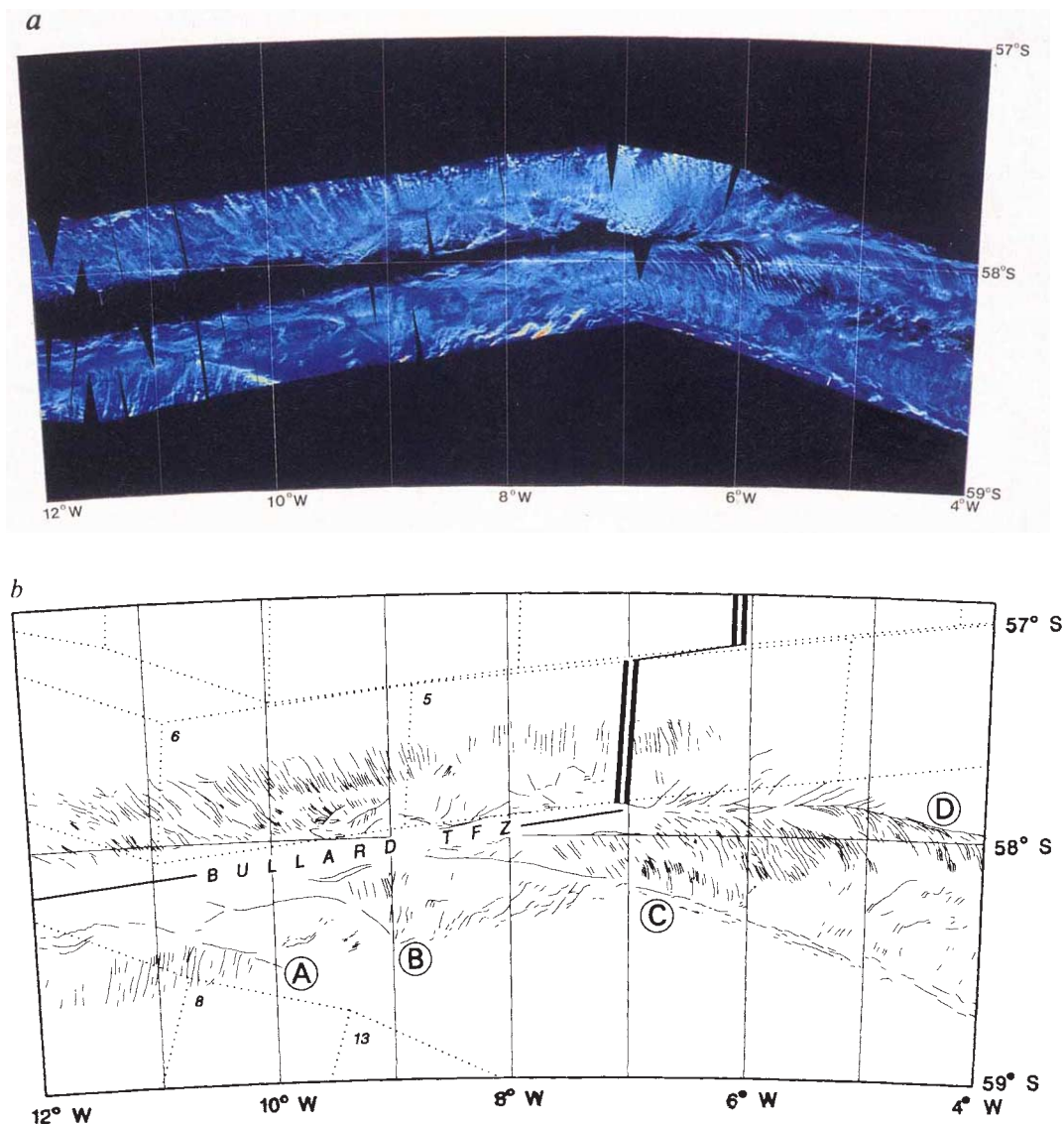


FIG. 1 Location of the Bullard fracture zone on the South American-Antarctic Ridge. Bathymetry from GEBCO sheet 5.16 is shown at 1,000-m intervals. Arrows represent SAM-ANT relative motion, with rates given in km Myr⁻¹,

according to the best-fitting vectors of De Mets *et al.*⁴. Dotted lines show the route of RRS *Charles Darwin* during cruise 37. Inset: principal plates in the SAAR region. Box defines the area shown in main figure.

FIG. 2 *a*, Digital mosaic of GLORIA images from the eastern end of the Bullard transform. Areas of highest acoustic backscattering are shown in orange and red; areas of lowest backscattering are dark blue. *b*, Line interpretation of GLORIA mosaic from around the Bullard transform. A-E denote fracture zones formed during sea-floor spreading before anomaly 6. Dotted lines are flow lines of SAM-ANT motion calculated by rotating the present ridge-transform intersections using the finite rotations of Barker and Lawver¹, and predicted isochrons obtained by rotating the present ridge segments. Small numerals indicate the magnetic anomaly numbers of these isochrons. See ref. 1 for details of the likely origin and age of crust to the east of fracture zone A, and its counterpart to the north. Note the change from WNW-ESE to W-E spreading at anomaly 6 (20 Myr).



sites in the deep oceans, particularly in association with propagating rifts and overlapping spreading centres on fast-spreading ridges¹⁴⁻¹⁶, and also with compressional intraplate deformation¹⁷⁻²⁰. These examples do not normally involve major transforms as in the present example, and tend to give rise to structures that turn in the direction of ridge offset. It is therefore difficult to see how such phenomena can account for the observed curvature.

Comparable deflections of structural trends have been observed in southern Cyprus, where a gradual clockwise rotation in strike of dykes in the sheeted complex of the Troodos Massif has been recorded^{21,22}, from N-S at a distance of 5-15 km north of the Arakapas fossil transform fault, to E-W close to the fault zone. Allerton²³ suggested that dyke rotation may have occurred very close to a ridge-transform boundary while the crust was still hot and weak. Similar structural rotations have been recorded in the Bay of Islands region of western Newfoundland^{24,25} and in northern Iceland²⁶.

Curvature of sea-floor fabric north of the Bullard transform is confined to crust older than ~10 Myr (west of ~9° W), indicating either that deformation has occurred a considerable time after emplacement or that the stress field changed at ~10 Myr. It also seems to be progressive, increasing westwards over a distance of many tens of kilometres, compared with less than 10 km in Troodos²⁷. This may represent progressive rotation

with distance along the transform, or a gradual reduction of shear stress with time.

The backscatter from sea-floor in the deformed zone north of the active transform is roughly what would be expected from crust of ~10 Myr, suggesting early deformation. If this occurred, as Allerton²³ suggests for southern Cyprus, by block rotation soon after emplacement (Fig. 3*b*), before the crust became too strong to deform by brittle failure, then it must have taken place earlier than ~10 Myr, when a change in the stress field or mode of extension occurred.

Extending this interpretation to the deformation of fabric between FZs C and D implies that a similar setting occurred before anomaly 6 (20 Myr BP) adjacent to FZ D. At that time, the direction of spreading was rotating anticlockwise, causing compression on dextral-offset transforms and a shortening of ridge segments. Deformation of young oceanic crust may then have occurred near the active transform corner of the ridge that intersected the dextral-offset FZ D. It has been suggested²⁶ that the existence of deformational shear zones may be associated with ridge jumps and the early stages of transform formation in older oceanic lithosphere. Such a setting would explain why deformation similar to that described here has not yet been seen elsewhere on the ocean floor. It might also explain the tectonic rotations in northern Iceland and in the Troodos ophiolite, both known to have been affected by ridge jumping.

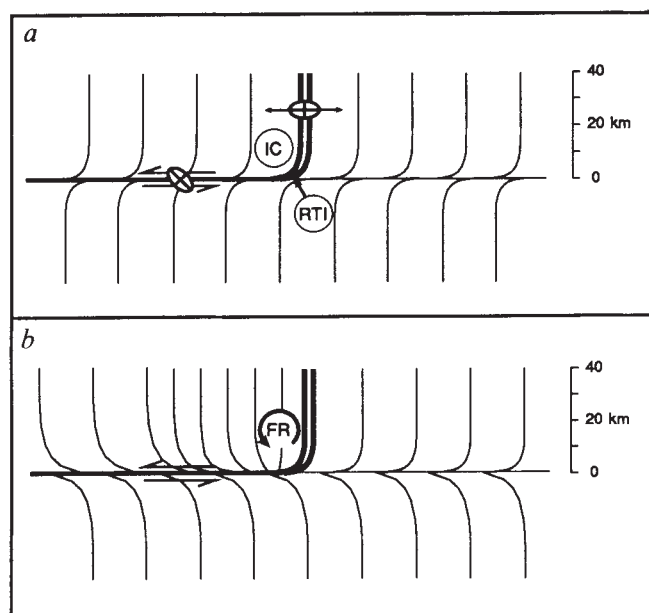


FIG. 3 *a*, Schematic diagram showing idealized tectonic fabric close to a fracture zone for a 'normal' stress field. Curvature is due to dyke injection and normal faulting in a stress field influenced by strike-slip. IC stands for transform or 'inside' corner; RTI stands for ridge-transform intersection. Major axes of ellipses show direction of minimum compressive stress⁷ on the spreading ridge and on the transform; arrows indicate relative plate motions. *b*, Schematic diagram showing idealized tectonic fabric for an 'anomalous' stress field in which deformation of young ocean crust occurs close to the RTI. In this simple model, it is assumed that similar deformation occurs at both RTIs, so that a symmetrical pattern results. FR stands for oblique-slip faulting and block rotation.

Extension by brittle deformation is more common on slow-spreading ridges such as the SAAR, where magma chambers may be absent for most of the time²⁸. Variations in the amounts of volcanism and tectonic extension have been observed on several sections of the Mid-Atlantic Ridge near transforms (for example, refs 29, 30), and even between spreading cells separated by nontransform offsets³¹. These variations could be cyclic, with tectonic extension assuming a particularly important role on slow-spreading ridges at times when volcanism is ineffective in relieving tectonic stresses. Near a long-offset, slow-slipping transform such as the Bullard transform, magma supply is likely to be further restricted owing to the distance from the centre of the spreading cell and to the cooling effect of the cold wall of lithosphere truncating the spreading ridge¹³. In such a setting, the imposition of transpressional stress is likely to produce the most profound tectonic deformation.

GLORIA imaging, with a resolution³² of 45–900 m, reveals little of the scale or mechanism of deformation. It seems likely that the curved fabric imaged here was formed by rotation of fault-bounded blocks³³, and, by analogy with northern Iceland²⁶ and southern Troodos³⁴, such blocks could have dimensions of hundreds of metres. Further work with higher resolution deep-towed sonars will be required to establish this. □

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Double cones as a basis for a new type of polarization vision in vertebrates

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MANY invertebrates^{1–4} and vertebrates^{5–14} are sensitive to the polarization of light. The biophysical basis of invertebrate polarization sensitivity is an intrinsic dichroism, the alignment of chromophores along the photoreceptor microvilli³. But such dichroism to axially propagating light is not present in vertebrate photoreceptors, whose chromophores are free to rotate in the plane of the outer-segment disc membranes, and a biophysical mechanism responsible for vertebrate polarization sensitivity has not been established. We hypothesize that the roughly elliptical cross-sectioned double-cone inner segment acts as a birefringent, polarization-sensitive dielectric waveguide, and that the double cone mosaic generates a 'polarization contrast' neural image. Here we confirm three predictions derived from these hypotheses: (1) 90° periodicity for polarization sensitivity; (2) polarization sensitivity maxima corresponding to the absolute orientation of the axes of the double-cone inner-segment cross-sections; and (3) action spectrum for polarization sensitivity corresponding to the absorption spectrum of the double cones. We also present evidence for a polarization-opponent neural encoding in vertebrates.

Vertebrate photoreceptors act as waveguides, trapping light initially in the inner segment and guiding it axially to the outer segment, where the photopigment molecules reside in stacked lamellar membranes¹⁵. Many species of fish, birds, amphibians and reptiles (but no mammals) have double cones, a distinct structural class of photoreceptors (see inset in Fig. 1). Micrographs of double-cone inner segments^{16,17} show the inner segments of the members of a double-cone pair to be contiguous. Thus, the double-cone inner segment can be expected to act as a unitary waveguide. The almost elliptical tangential cross-section of the double-cone inner segment may endow the waveguide with some degree of geometrical birefringence, that

is to enable it to trap and propagate light of one polarization more efficiently than others to the outer segments. Such geometrical birefringence is exhibited by elliptically cross-sectioned optical fibres¹⁸. Moreover, the effective dimensions of the waveguide cross-section are probably decreased by the graded distribution of material density revealed in electron micrographs, which show the mitochondria to be most densely concentrated along the common border of the inner segments of the double-cone pair (S. S. Easter, personal communication).

The hypothesis that the double-cone inner segment acts as a birefringent waveguide apparently predicts that polarization

sensitivity would show a 180° periodicity, such as previously found¹². The double cones in many teleosts, however, are known to be arranged in square-patterned tetradic mosaics in which the long axes of adjacent double-cone inner segment cross-sections are locally orthogonal^{19,22}. This mosaic arrangement, combined with the birefringent waveguide hypothesis predicts a 90° periodicity for local polarization sensitivity. This prediction

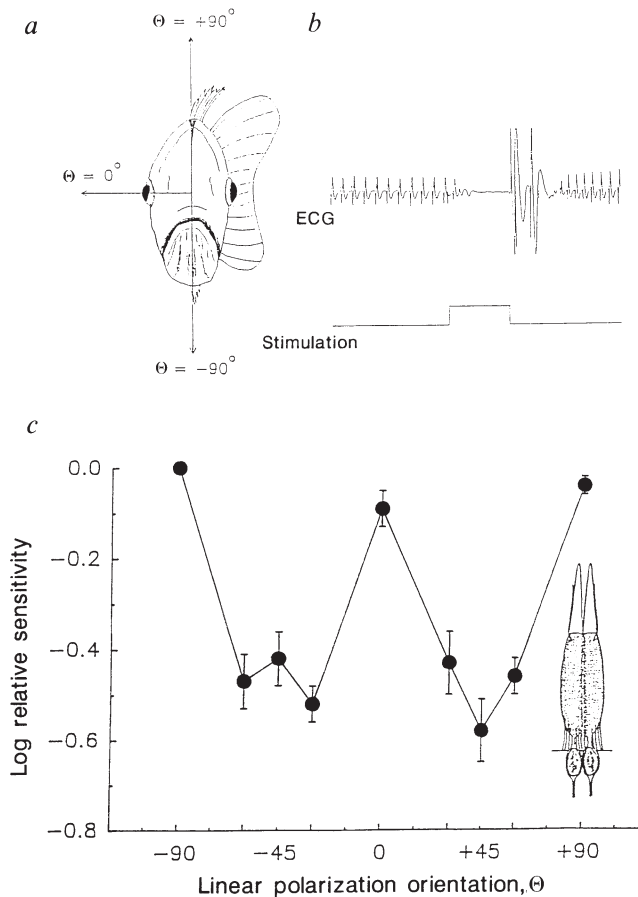


FIG. 1 *a*, Head-on illustration of a green sunfish (*Lepomis cyanellus*). The linear polarization orientation (Θ) is defined relative to the body axis. *b*, Electrocardiogram (ECG) record illustrating a positive response, defined generally as a full-amplitude spike interval at least 1.5 times longer during the 5-s stimulus presentation than during the 10 s before stimulus onset. *c*, Photopic thresholds for stimuli linearly polarized at different orientations, Θ (Wratten gel filter 23A; dominant wavelength 604 nm). Each fish was tested at each Θ three times over several days. All data points are means $\pm$ 1 s.e.m. ($n=3$ fish). Inset shows a line drawing by Engstrom¹⁶ of a double (twin) cone closely resembling those in *L. cyanellus*. METHODS. Green sunfish (Fender's Fish Hatchery, Baltic, Ohio, kept on a 12 h–12h light–dark cycle), were classically conditioned to suppress heart rate^{12,27,28} during the presentation in Maxwellian view of visual stimuli in the fronto-parallel plane imaged onto the temporal retinae of both eyes. All stimuli subtended 12.5° of visual angle at the corneas. Heart rates were recorded in a movement-restricting opaque black plastic box (with a frontal port for the light beam) with two Ag/AgCl pellet electrodes external to the animal²⁹, and the resulting ECGs were amplified and bandpassed. Heart beat spike amplitudes were typically 50–250 μ V at the electrodes. Intertrial intervals randomly ranged between 30 and 60 s. All of the behavioural experiments measured luminance thresholds with an intensity staircase scheme¹² in which subthreshold stimuli were intensified in 0.2 log unit steps until the animal gave a positive response to two consecutive stimuli. The intensity which elicited the first response in this sequence was taken to be threshold for that experimental condition.

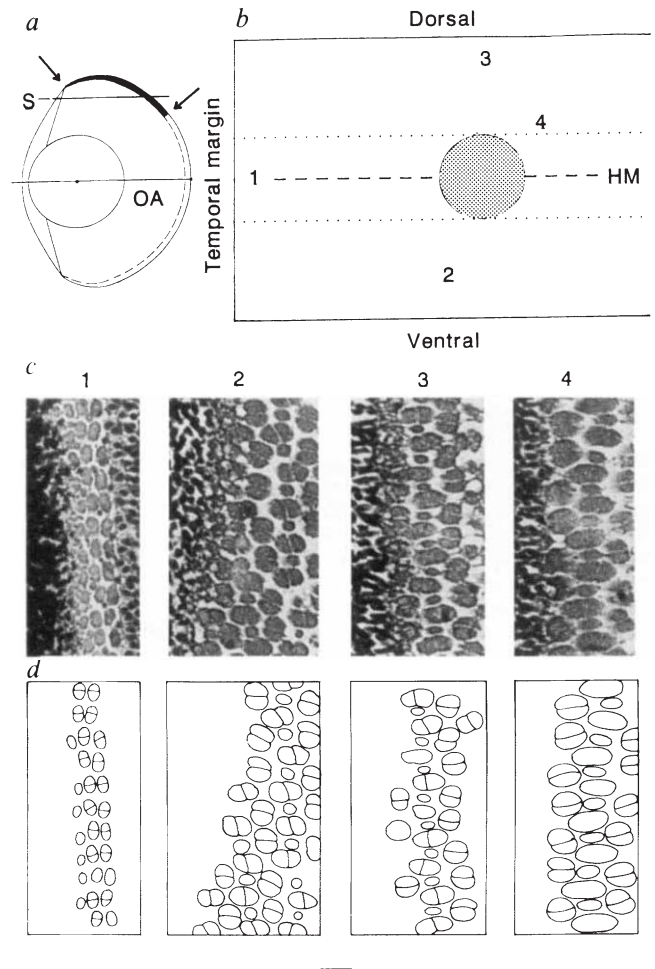


FIG. 2 *a*, Line drawing of a fish right eye in horizontal section (after ref. 30), indicating the extent along the horizontal meridian along which the visual stimuli were imaged (between the arrows). The line 'S' indicates a typical plane of section used in the anatomical analysis. OA, Optic axis. *b*, Two-dimensional diagram of the horizontal strip of temporal retina indicated in *a*, showing the horizontal meridian (HM) and the positions of the four retinal sections in *c*. The measured fishes' eye movements resulted in the 12.5° diameter circular stimulus being imaged somewhere along the 'band' of retina defined by the dotted lines (hatched area shows the modal position of the 12.5° diameter stimulus; $1^\circ \approx 84 \mu$ m in the average sized eye). *c*, Photographs of portions of 4 μ m-thick tangential sections of temporal retinas, oriented with respect to, and identified by, their retinal positions (1–4) in *b* (scale bar, 10 μ m). Section 1 shows the photoreceptor mosaic near the retinal margin where the double cones are aligned parallel to one another and to the margin tangent, as reported for another species¹⁹. Sections 2–4 feature square-patterned mosaics of double-cone inner segments. In tangential sections of whole mounts we found the double-cone inner segments to be almost elliptical, with a 2:1 ratio between the major and minor axes. The axis ratios in each section in *c* are distorted by the oblique sectioning; the 'slit' between the members of a double-cone pair uniquely defines the minor axis. *d*, Line drawings of the double-cone inner segment cross-sections in the micrographs in *c*. Scale bar, 10 μ m. METHODS. Fully light adapted fish eyes were fixed in 3.6% glutaraldehyde in 0.1 M PO_4 buffer, dehydrated and embedded in paraffin. Planes of section (see 'S' in *a*) were taken parallel to the optic axis ('OA' in *a*); all sections were mounted and haematoxylin-eosin stained.

assumes that at threshold the signals from adjacent, orthogonally oriented double cones are linear, and are combined linearly.

We determined the local polarization sensitivity of green sunfish (*Lepomis cyanellus*) with a classical conditioning technique, in which fish learned to associate a visual stimulus (conditioned stimulus) with a mild tail shock (unconditioned stimulus); the unconditioned response is a heart-rate suppression (Fig. 1b). In all experiments presented here, the target was directly in front of the fish, and subtended 12.5° of visual angle. Figure 1c shows light-adapted thresholds for linearly polarized, long-wavelength targets. The fish were maximally sensitive to horizontal (H) and vertical (V) polarizations (defined with respect to the fish body axis; Fig. 1a), and up to 0.6 log units less sensitive to light linearly polarized at oblique orientations. The threshold profile is a W-shaped function of polarization orientation (Θ) with clear 90° periodicity.

Given the result in Fig. 1c, the birefringent waveguide hypothesis makes an unequivocal prediction about the absolute orientation of the double-cone inner segments in the region of the retina stimulated by the target, that is that their symmetry axes should be aligned with the dorsal-ventral and medial-lateral axes of the fish's body. Figure 2 shows a confirmation of this prediction: the axes of double-cone cross-sections near the horizontal meridian in the temporal retina are aligned as predicted.

Both members of an *L. cyanellus* double-cone pair contain the same photopigment ($\lambda_{\max} = 621$ nm), and that no single cones ever contains this pigment²³. Cones and rods sensitive to middle wavelength (the only other photoreceptor classes in the adult *L. cyanellus* retina²³) are always circularly cross-sectioned, single receptors. These facts lead to a third prediction of the double-cone waveguide hypothesis: polarization sensitivity should be restricted to the adaptation conditions and the spectral region governed by the double cones.

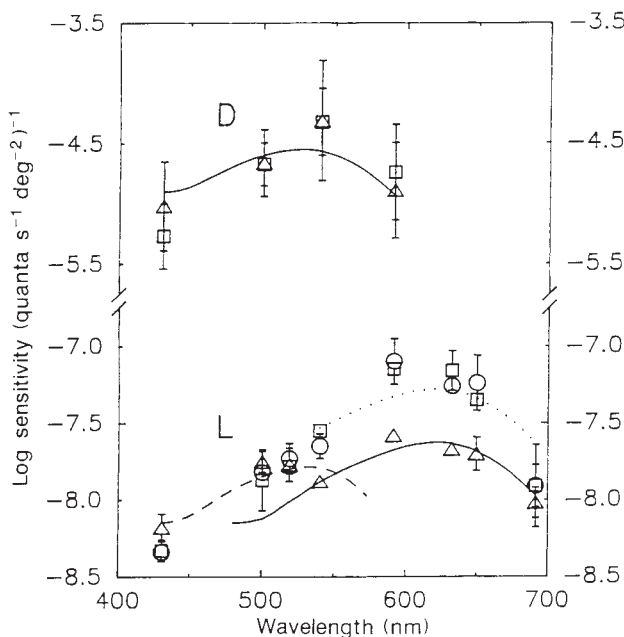


FIG. 3 Threshold spectral sensitivities under fully dark adapted (D) and light adapted (L) conditions for monochromatic stimuli (10 nm bandpass) of various linear polarizations Θ ($\circ$, 0°; Δ , -45°; $\square$, -90°, as in Fig. 1c). Uncorrected absorbance spectra of the three *L. cyanellus* photopigments²³ have been fit to the data by least squares (vertical sliding only), indicating which photoreceptor class governs threshold in each spectral region. The illustrated absorbance spectra are, for the light-adapted condition, from the middle-wavelength- (dashed line) and long-wavelength-sensitive (solid and dotted lines) cone photopigments, and for the dark adapted condition, the rod photopigment (solid line).

Figure 3 confirms this third prediction. In the fully light-adapted state, differential sensitivity to polarization is restricted to wavelengths > 540 nm; here the action spectrum corresponds reasonably well with the α -band of the long-wavelength sensitive cone pigment measured by microspectrophotometry²³. In contrast, the fish exhibited no polarization sensitivity in either the region < 540 nm under light-adapted conditions, where the action spectrum is probably that of the middle-wavelength cones, nor under fully dark-adapted conditions, where the action spectrum is clearly that of the rods²³. Although polarization sensitivity arises only from the activity of double cones, the action spectrum is not predicted quantitatively by a Beer's Law model and, moreover, the differential sensitivity to polarization declines both at 542 nm and at 691 nm, suggesting that the waveguide property may alter the *in situ* absorption spectrum. The observation that sunfish have no differential sensitivity to polarization under dark-adapted conditions (compare the thresholds under the two adaptation conditions at 590 nm) rules out the hypothesis that *L. cyanellus*' rods can support polarization

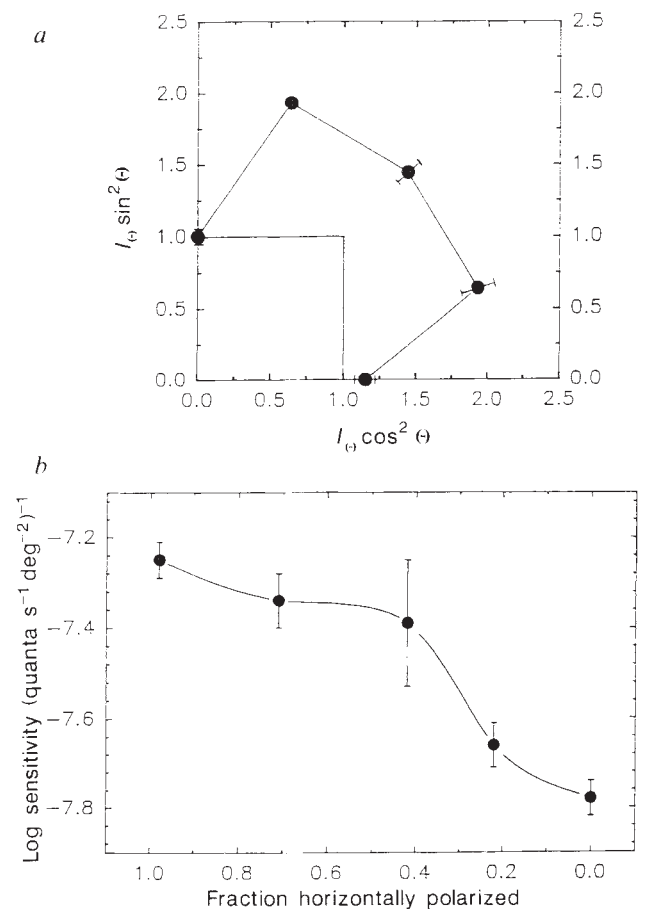


FIG. 4 a, The data from Fig. 1c replotted as mixtures of two orthogonal polarization components H (horizontal) and V (vertical); data are linearly scaled and normalized by the mean threshold for the V component alone. The unit square represents the region wherein thresholds would be expected to lie if they arose from same-signed combinations of signals from the two classes of double cones optimally tuned to the H and V components, respectively. b, Light-adapted sensitivity to monochromatic stimuli (632.8 nm) as a function of net horizontal polarization. The fractional polarizations were produced by passing horizontally linearly polarized light through a calibrated, rotatable quarter-wave retardation plate rated at 632.8 nm. The sensitivity to stimulus '0.0' on the abscissa, representing right-handed circularly polarized light, is statistically equivalent to that for 632.8 nm linearly polarized light at orientation $\Theta = -45^\circ$ under light-adapted conditions (Fig. 3).

vision, and also rejects any hypothesis which ascribes polarization sensitivity to some dichoric or birefringent element of the eye's preretinal optical apparatus common to all photoreceptors under all adaptation conditions²⁴.

The 90° periodicity of polarization sensitivity (Fig. 1c) also argues against any single preretinal dichroic filter which would reside distal to all the cones sensitive to long wavelength light, as such a filter would be expected to produce a 180° periodicity.

Given the hypothesis that double cones function as polarization-sensitive waveguides Fig. 1c reveals another fundamental property of the neural signals generated by the double-cone mosaic. By a physical identity, a linearly polarized light can be uniquely described as the vector sum of two orthogonally polarized components. In Fig. 4a the thresholds of Fig. 1c are replotted as their unique horizontal and vertical components. If the signals generated by receptors with maximal sensitivity to the purely H-polarized and purely V-polarized stimuli were transmitted independently, the thresholds would be expected to lie near the unit square; if the signals were combined linearly with the same sign, the thresholds would lie between the unit circle and the negative diagonal. In fact, the thresholds for stimuli comprising both H and V polarization components were always greater than thresholds for the purely horizontally or purely vertically polarized stimuli; for example, admixing a V-component at 50% of its own threshold to an H-polarized stimulus requires that the intensity of the latter be doubled to reach threshold. As any increase in light intensity must necessarily increase the quantal absorption rate of all the cones under the image, the threshold points lying outside the unit square in Fig. 4 establish the presence of a polarization-opponent neural mechanism. This result suggests, by analogy with the role of colour-opponent coding²⁵, that polarization vision in this and related vertebrates serves as a contrast-detecting system.

Stimuli with reduced fractions of H polarization were therefore used to measure a 'polarization contrast sensitivity' function for the sunfish. The photopic sensitivity to H-polarized light decreased as the net percentage of light polarized in that plane was reduced (Fig. 4b). The thresholds for all targets with polarization contrasts $\geq 20\%$, however, are reliably lower than the threshold for a circularly polarized target, which has zero polarization contrast. We conclude that the polarization-opponent neural code is capable of mediating the detection of targets with polarization contrast $\geq 20\%$.

Perhaps the most striking general conclusion to be drawn from our results is that the polarization contrast mechanism we have inferred in the sunfish is the most sensitive visual channel available to the animal for detecting small targets under our light-adapted conditions, in the long-wavelength region of the visible spectrum (the region where underwater scattering is least contrast-degrading²⁶). Our 12.5° stimulus, for example, subtends the same visual angle as does a sunfish at 0.5 m, whose scales we have found (unpublished observations) to partially polarize reflected light. Polarization contrast thus seems likely to be used by this vertebrate (and the many others possessing similar double-cone mosaics) as a mechanism for object detection. Certainly, a polarization contrast visual system will improve the visibility of objects that partially polarize reflected light in an environment that has much scattered light with random polarization. □

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Dopamine activation of the arachidonic acid cascade as a basis for D₁/D₂ receptor synergism

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UNDERSTANDING the actions of the neurotransmitter dopamine in the brain is important in view of its roles in neuropsychiatric illnesses¹. Dopamine D₁ receptors, which stimulate both adenylyl cyclase² and phospholipase C³, and D₂ receptors, which inhibit them^{4,5}, can nevertheless act synergistically to produce many electrophysiological and behavioural responses⁶. Because this functional synergism can occur at the level of single neurons, another, as yet unidentified, signalling pathway activated by dopamine has been hypothesized⁷. We report here that in Chinese hamster ovary (CHO) cells transfected with the D₂ receptor complementary DNA, D₂ agonists potently enhance arachidonic acid release, provided that such release has been initiated by stimulating constitutive purinergic receptors or by increasing intracellular Ca²⁺. In CHO cells expressing D₁ receptors, D₁ agonists exert no such effect. When D₁ and D₂ receptors are coexpressed, however, activation of both subtypes results in a marked synergistic potentiation of arachidonic acid release. The numerous actions of arachidonic acid and its metabolites in neuronal signal transduction⁸ suggest that facilitation of its release may be implicated in dopaminergic responses, such as feedback inhibition mediated by D₂ autoreceptors, and may constitute a molecular basis for D₁/D₂ receptor synergism.

The effect of D₂ receptor stimulation on the release of arachidonic acid (AA) from membrane phospholipids was studied in CHO cells, transfected with rat D₂ receptor cDNA (CHO(D₂)) and labelled by incubation with [³H]AA (Fig. 1). The D₂ receptor agonist quinpirole did not affect [³H]AA release when applied alone (Fig. 1; Table 1). By contrast, the drug strongly potentiated [³H]AA release when this was evoked by stimulating purinergic receptors with ATP (1–100 μM; Fig. 1a)⁹. Potentiation of ATP-induced [³H]AA release was half-maximal at 34 ± 3 nM quinpirole (mean ± s.e.m., n = 4) and maximal at 250 nM (Fig. 1b). Because the stimulation of constitutive

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purinergic receptors produced a transient increase in intracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$ (effector concentration for half-maximal (EC_{50}) at $5 \mu\text{M}$, as determined with Fura-2), we examined the effects of quinpirole on the release of $[\text{^3H}]\text{AA}$ evoked by the Ca^{2+} ionophore A23187. Potentiation of A23187-induced $[\text{^3H}]\text{AA}$ release was half-maximal at $30 \pm 10 \text{ nM}$ quinpirole, and maximal at 50 nM (Fig. 1b). Similarly, quinpirole enhanced the release of $[\text{^3H}]\text{AA}$ evoked either by α -thrombin, which enhances $[\text{Ca}^{2+}]_i$ and activates phospholipase A_2 (PLA_2) in CHO by a receptor-mediated mechanism⁹, or by ionomycin (not shown). By contrast, D_2 receptor stimulation did not affect either A23187-induced formation of $[\text{^3H}]\text{inositol}$ phosphates, catalysed by phosphatidylinositol-specific phospholipase C (ref. 10) or A23187-induced release of $[\text{^3H}]\text{choline}$, catalysed by both phospholipases C and D (ref. 11) (not shown).

Potentiation of $[\text{^3H}]\text{AA}$ release by quinpirole resulted from occupation of D_2 receptors. In agreement with this is: (1) the response was antagonized by the D_2 receptor blocker haloperidol (Fig. 1c); (2) dopamine as well as other D_2 receptor agonists enhanced A23187-induced $[\text{^3H}]\text{AA}$ release ($\text{EC}_{50} = 14 \pm 2 \text{ nM}$ for dopamine ($n = 8$) and $6 \pm 1 \text{ nM}$ for pergolide ($n = 16$)); (3) the D_1 receptor agonist SKF 38393 was ineffective (Fig. 1b);

and (4) wild-type CHO cells did not respond to quinpirole plus ATP (Table 1) or to quinpirole plus A23187 (not shown).

The ability to affect $[\text{^3H}]\text{AA}$ release was selective for the D_2 receptor subtype. In CHO clones expressing D_1 receptors, neither SKF 38393 nor quinpirole affected ATP-induced $[\text{^3H}]\text{AA}$ release (Table 1). But SKF 38393 did enhance the accumulation of cyclic AMP (from 0.8 ± 0.1 to $11.3 \pm 0.9 \text{ pmol per well}$, $n = 4$), indicating that D_1 receptors were coupled to adenylyl cyclase. In addition, only a small effect of quinpirole (blocked by haloperidol) was seen in CHO cells expressing human D_3 receptor¹² (Table 1). D_3 receptors are not (or only weakly) linked to adenylyl cyclase inhibition when expressed in CHO (ref. 13 and our unpublished results), suggesting that the appropriate G protein may be lacking in these transfected clones.

Inhibition of adenylyl cyclase activity by D_2 receptors often involves a G-protein member of the G_i/G_o family, and is inhibited by pertussis toxin (PTX)⁵. Similarly, in CHO(D_2) cells, PTX prevented the response to quinpirole without affecting A23187-induced AA release (Fig. 1d), indicating that a PTX-sensitive G protein, linked to the D_2 receptor, may regulate AA release (possibly by enhancing the sensitivity of PLA_2 to intracellular Ca^{2+})¹⁴.

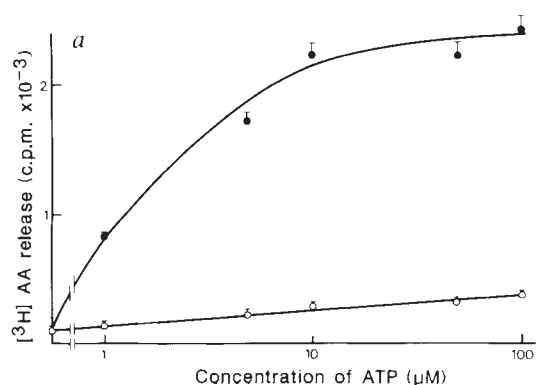
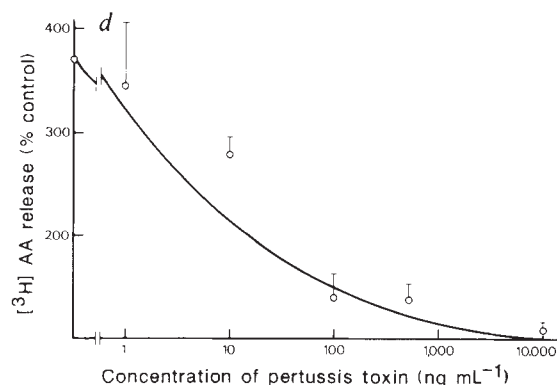
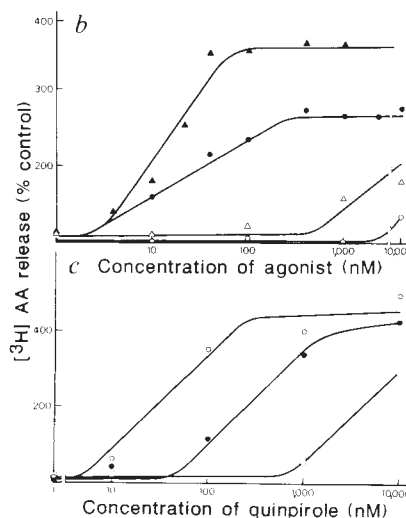


FIG. 1 Quinpirole facilitates $[\text{^3H}]\text{AA}$ release from prelabelled CHO(D_2) cells. **a**, Effect of quinpirole on the release of $[\text{^3H}]\text{AA}$ evoked by stimulating constitutive purinergic receptors with ATP. Cells were incubated with ATP (1–100 μM) in the absence (○) or in the presence of quinpirole at 0.5 μM (●). When applied alone, 100 μM ATP stimulated $[\text{^3H}]\text{AA}$ release to $343 \pm 14\%$ of control ($P < 0.05$, Student's t -test). **b**, Concentration-response curves for the potentiating effect of quinpirole on $[\text{^3H}]\text{AA}$ release evoked either by ATP (100 μM) (●) or by Ca^{2+} ionophore A23187 (4 μM) (▲). The figure also demonstrates the lack of effect of SKF 38393, a D_1 receptor agonist, on ATP-induced (○) or A23187-induced (△) $[\text{^3H}]\text{AA}$ release. The small response at 10 μM SKF 38393 was probably due to the stimulation of D_2 receptors, because it was blocked by the selective D_2 receptor antagonist, raclopride (0.5 μM). **c**, Haloperidol inhibits the potentiation by quinpirole of A23187-evoked $[\text{^3H}]\text{AA}$ release. CHO(D_2) cells were incubated with quinpirole (1 nM–10 μM) plus A23187 (4 μM), without (○) or with haloperidol at 10 nM (●) or 100 nM (□). **d**, Potentiation by quinpirole of A23187-evoked $[\text{^3H}]\text{AA}$ release is prevented by incubating CHO(D_2) cells with pertussis toxin (PTX). Cells were incubated for 4.5 h with various concentrations of PTX (1–10,000 ng ml^{-1}) before exposing them to quinpirole (1 μM) plus A23187 (4 μM).

METHODS. Transfection of CHO cells with rat D_{2A} receptor (also termed D_{2L})²⁷ cDNA has been described elsewhere¹³. Wild-type and transfected CHO clones were maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. CHO(D_2) cells expressed 1.3×10^5 D_2 receptors per cell. Cells (24-well plates) were labelled by incubation with $[\text{^3H}]\text{AA}$ (Amersham, 219 Ci mmol^{-1} , 0.5 $\mu\text{Ci ml}^{-1}$) in DMEM (1 ml) containing 0.5% BSA for 2 h at 37 °C. To eliminate unincorporated radioactivity, cells were washed twice with 0.5 ml DMEM plus BSA before incubating them for 30 min at 37 °C in 1 ml of the same medium, containing final concentrations of the appropriate drugs. $[\text{^3H}]\text{AA}$ release was determined by liquid scintillation counting in samples (0.5 ml) of the incubation medium. No significant difference in $[\text{^3H}]\text{AA}$ labelling of cell lipids was observed among CHO clones in any experiments. Incubation with PTX



did not affect $[\text{^3H}]\text{AA}$ labelling. Analysis by thin-layer chromatography²⁸ revealed that free $[\text{^3H}]\text{AA}$ constituted more than 90% of the released radioactivity in both control and stimulated samples. Results represent means $\pm$ s.e.m. of 8–12 separate determinations. In **b** and **c**, s.e.m. bars are omitted for clarity. In each experiment, results were calculated as percentage of either A23187-treated or ATP-treated cells. $[\text{^3H}]\text{AA}$ release from CHO(D_2) cells incubated with 4 μM A23187 was $672 \pm 47 \text{ c.p.m.}$ ($n = 72$) and for cells incubated with 100 μM ATP $302 \pm 25 \text{ c.p.m.}$ ($n = 32$). Control cells released $105 \pm 5 \text{ c.p.m.}$ of $[\text{^3H}]\text{AA}$ ($n = 80$). Curves were fitted to the experimental data using the least squares method.

In agreement with their opposing effects on adenylyl cyclase activity, D_1 and D_2 receptors have antagonistic roles in the regulation of some neural functions⁶. In many cases, however, these receptors also exert synergistic actions⁶. In the striatum for example, where about 30% of neurons may express both subtypes¹⁵, there is a synergistic inhibition of $(Na^+ + K^+)$ ATPase activity by D_1 and D_2 receptor coactivation⁷.

To examine whether D_1 and D_2 receptors may interact in regulating $[^3H]AA$ release, we used CHO cells transfected with both receptor cDNAs (CHO($D_1 + D_2$)). In these cells, which express 10 times more D_2 than D_1 receptor, the overall effect of D_1/D_2 receptor stimulation by dopamine was to enhance cAMP formation (Fig. 2c), as previously shown in striatal slices¹⁶. This response was blocked by the D_1 antagonist SCH 23390, while an underlying D_2 -dependent inhibition was evidenced using the D_2 -receptor blocker raclopride (Fig. 2c).

In CHO($D_1 + D_2$) cells, quinpirole potentiated A23187-evoked release of $[^3H]AA$, whereas SKF 38393 was ineffective (Fig. 2a). Response to quinpirole was strongly enhanced, however, in the presence of 0.5 μM SKF 38393 (Fig. 2a) (an effect which was half-maximal at 30 nM, and maximal at 0.5 μM , not shown). Dopamine, which acts both on D_1 and on D_2 receptor subtypes, enhanced A23187-induced $[^3H]AA$ release in CHO($D_1 + D_2$) cells with seven-fold greater potency than in CHO(D_2) cells (EC_{50} in CHO($D_1 + D_2$) was 2 ± 0.2 nM versus 14 ± 2 nM in CHO(D_2), $n = 8$) (Fig. 2b). The greater potency of dopamine may be attributed to a synergistic contribution of D_1 receptors to the D_2 response. In agreement, raclopride inhibited completely the response to dopamine, whereas SCH 23390 reduced its potency by about 10-fold (Fig. 2b). In the presence of SCH 23390, the EC_{50} for dopamine was 28 ± 4 nM, a value similar to that obtained in CHO(D_2) cells.

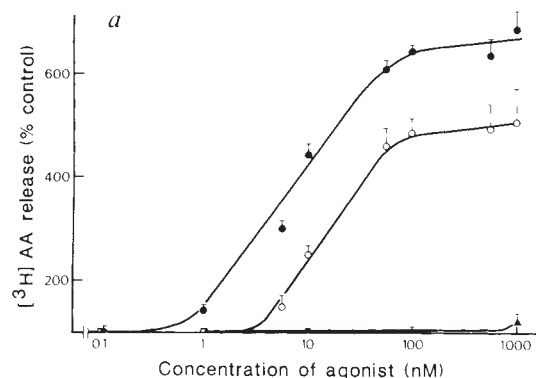


TABLE 1 Effects of D_1 and D_2 receptor agonists on basal or ATP-induced $[^3H]AA$ release in transfected CHO cells

Cell	ATP + quinpirole	Quinpirole (1 μM)	SKF 38393 (1 μM)	ATP (25 μM)	ATP + SKF 38393
CHO (wild type)	112 $\pm$ 13	90 $\pm$ 7	87 $\pm$ 16	111 $\pm$ 2	114 $\pm$ 7
CHO (D_1)	106 $\pm$ 19	68 $\pm$ 8	78 $\pm$ 19	114 $\pm$ 16	141 $\pm$ 19
CHO (D_{2A})	330 $\pm$ 93*	90 $\pm$ 23	88 $\pm$ 22	119 $\pm$ 10	131 $\pm$ 19
CHO (D_{2B})	267 $\pm$ 34*	90 $\pm$ 14	103 $\pm$ 24	142 $\pm$ 13	ND
CHO (D_3)	178 $\pm$ 3*	103 $\pm$ 5	102 $\pm$ 2	142 $\pm$ 8	139 $\pm$ 5

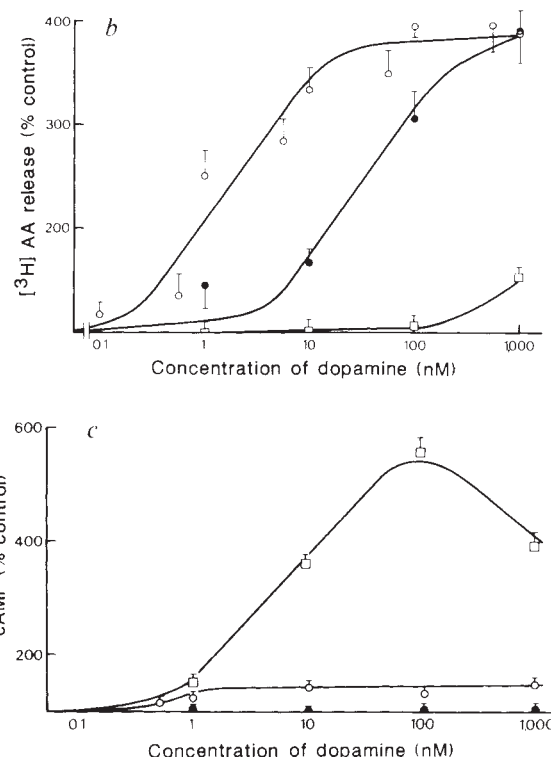
Transfections were done as described in Fig. 1 legend. Receptor capacities for the transfected clones (determined in binding experiments described in refs 25 and 26) were the following (receptor/cell): CHO(D_1), 8×10^3 ; CHO(D_{2A}), 1.1×10^5 ; CHO(D_{2B}), 1.4×10^5 ; CHO(D_3), 1×10^5 . $[^3H]AA$ labelling and incubation with drugs were done as described (Fig. 1 legend). In these experiments, the concentration of ATP used (25 μM) produced only a weak stimulation of $[^3H]AA$ release which, in the absence of quinpirole, was not statistically significant. Similar results were obtained, however, when 100 μM ATP or 4 μM A23187 were used (data not shown). Results represent the mean $\pm$ s.e.m. of at least four separate determinations, and are expressed as percentage of unstimulated controls. In control conditions, wild-type CHO cells released 174 ± 8 cpm of $[^3H]AA$ and similar control release was obtained in all clones tested (not shown). $[^3H]AA$ release was not statistically different from control samples unless so indicated.

* Significantly different from both control and ATP-treated samples. $P < 0.05$ (Student's t -test).

Two observations suggest that cAMP formed through D_1 receptor stimulation may participate in the synergistic AA response. First, in CHO($D_1 + D_2$) cells the overall effect of dopamine was to enhance cAMP formation (Fig. 2c). Second, in CHO(D_2) cells the cAMP analogue 8-bromo-cAMP (0.1 mM) enhanced by $272 \pm 43\%$ ($n = 4$) the effect of 1 μM quinpirole on $[^3H]AA$ release (evoked by 4 μM A23187). Interestingly, the synergistic inhibition of $(Na^+ + K^+)$ ATPase activity in striatal neurons by D_1 and D_2 receptor stimulation may also involve

FIG. 2 D_1 and D_2 act synergistically on $[^3H]AA$ release, and antagonistically on cAMP formation. *a*, Concentration-dependent effects of quinpirole (○), SKF 38393 (▲) and of quinpirole plus a fixed concentration of SKF 38393 (0.5 μM) (●) on $[^3H]AA$ release evoked by A23187 (4 μM) from CHO($D_1 + D_2$) cells. *b*, Concentration-response curves for the actions of dopamine (○), dopamine plus the D_1 receptor antagonist SCH 23390 (1 μM) (●) and dopamine plus the D_2 receptor antagonist raclopride (1 μM) (□) on A23187-induced $[^3H]AA$ release. *c*, Dopamine stimulates cyclic AMP accumulation in CHO($D_1 + D_2$) cells. Cells were incubated with dopamine (○), dopamine plus SCH 23390 (1 μM) (●) or dopamine plus raclopride (1 μM) (□) in the presence of A23187 (4 μM), before measuring cAMP by radioimmunoassay in samples of lysed cells.

METHODS. The CHO(D_2) cells used in Fig. 1 were cotransfected using Lipofectin (GIBCO) with a pCD-BS plasmid containing the D_1 receptor gene (gift of P. Seeman) and a pUT 523 plasmid (CAYLA, Toulouse, France) containing a phleomycin-resistance gene. Cells were selected in DMEM containing 50 μg ml^{-1} phleomycin and clonal cell lines screened in binding experiments with ^{125}I -labelled SCH 23982 (NEN), a selective D_1 receptor radioligand²⁶. CHO($D_1 + D_2$) cells used in these experiments expressed about 1.2×10^4 D_1 receptors per cell. Expression of D_2 receptor was not affected by D_1 receptor cotransfection. $[^3H]AA$ release was determined as described in the legend to Fig. 1. Accumulation of cAMP was determined in the same



cultures used for the $[^3H]AA$ release experiments depicted in Fig. 2b. The cAMP was extracted from cells using 0.1 M HCl and sonication, and cAMP concentration determined using a radioimmunoassay kit (Amersham) after neutralization of the acid extracts. Results represent the means $\pm$ s.e.m. of 4–12 separate determinations. The cAMP levels are expressed as percentage of A23187-treated cells (1.46 ± 0.08 pmol per well, $n = 8$). A23187 did not significantly affect cAMP levels (not shown).

cAMP⁷. As AA and its cytochrome P₄₅₀ metabolites are potent inhibitors of (Na⁺ + K⁺) ATPase¹⁷, their formation may account for the synergism described in striatal neurons.

We have shown here that D₂ agonists enhanced Ca²⁺-stimulated AA release at nM concentrations and with EC₅₀ 20- to 30-fold lower than the corresponding K_i for receptor binding¹³. This suggests that partial D₂ receptor occupation is sufficient to produce a robust AA response. It will be important to determine whether this facilitatory action, described here for transfected CHO cells, occurs in brain areas, such as the striatum, where comparable D₂ receptor densities are found and receptor-stimulated AA release has been demonstrated¹⁸.

The facilitatory effect of D₂ receptors on Ca²⁺-dependent AA release reported here suggests that, in presynaptic terminals, the

Ca²⁺ increase which follows depolarization and triggers dopamine release may, at the same time, enable D₂ autoreceptors¹⁹ to release AA and its metabolites. These may, in turn, participate in the autoinhibitory actions of dopamine, for example by modulating K⁺ or Ca²⁺ channel activities²⁰⁻²³ or protein phosphorylation²⁴. Finally, as costimulation of a Ca²⁺-mobilizing receptor revealed an unexpected amplification by dopamine of AA release, more neurotransmitters than previously thought may operate through the AA cascade by a similar mechanism.

Note added in proof: A study by Kanterman *et al.*²⁹ has appeared after submission of the present study, showing that stimulation of D₂ receptors in transfected CHO cells augments A23187-induced [³H]AA release. □

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A role for peptide in determining MHC class II structure

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T LYMPHOCYTES recognize antigen-derived peptides associated with major histocompatibility complex (MHC) class I or class II proteins^{1,2}. Peptide is critical in class I heavy-chain folding and/or stable association with β_2 -microglobulin³⁻⁶. Although data exist suggesting a relationship between class II structure and peptide association⁷⁻⁹, no equivalent positive contribution of peptide to the folding state or stability of class II dimers has yet been demonstrated. We report here that most purified E α^k E β^k molecules leaving low pH in the absence of specific peptide lack a compact, stable dimeric structure. Brief exposure to the appropriate peptide just before and during neutralization promotes this specific conformation in proportion to stably bound peptide, indicating that peptide is important in determining class II MHC structure. Our results also indicate that efficient generation of long-lived peptide-class II complexes involves two stages: initial peptide binding in an acidic environment, which enhances the ability of class II to enter a conformation, from which stabilization upon neutralization results in high-affinity binding of previously associated peptide.

When analysed without reduction or boiling, class II molecules show three distinct forms on electrophoresis in SDS-polyacrylamide gels⁸: 'compact' (C) dimers migrating with an apparent relative molecular mass of 56,000 (M_r , 56K), 'floppy' (F) dimers (63-67K) which are partially denatured C dimers, and disassembled α (31-33K) and β (28-30K) chains. To investigate the relationship between peptide binding and the

TABLE 1 Specificity of the peptide effect on E α^k E β^k conformation

E α^k E β^k incubation conditions	Relative protein density*			
	Floppy	Compact	E α	E β
pH 4.5, no peptide → pH 7.2	49.0	19.0	8.0	5.0
pH 4.5 + DASP → pH 7.2	23.0	48.0	2.0	3.0
pH 4.5 + OVA 323-339 → pH 7.2	60.0	14.0	4.0	6.0
pH 4.5 + HEL 46-61 → pH 7.2	48.0	20.0	7.0	3.0

*SDS-polyacrylamide gel stained with silver was scanned for protein density. Results for each E α^k E β^k form are expressed as per cent of total protein in the applied sample, as determined by laser densitometry. Less than 25% of total E α^k E β^k appears as high molecular weight bands (>90K) in every sample. Experimental conditions for treatment and gel analysis are as described in the legend to Fig. 1b. Peptides were used at 100 μ M. All peptides were shown to have appropriate bioactivity by stimulation of T-cell hybridomas in the context E α^k E β^k (DASP), A α^d A β^d (OVA 323-339), and A α^k A β^k (HEL 46-61).

structure of class II, we isolated E α^k E β^k molecules⁷ and analysed their gel migration under a variety of conditions. Most purified E α^k E β^k incubated at pH 7.2 with or without added peptide migrates as C dimers of 56K; a small fraction (5-10%) migrates as free α and β chains (Fig. 1a and b). Incubation for 30 min at pH 4.5 and 37 °C, followed by neutralization, converts most of the C dimers into either F dimers or free α and β chains. These α and β chains derive from SDS dissociation of 'unstable' (U) dimers, based on co-precipitation by anti-C terminal peptide antiserum (data not shown). The presence during acid treatment and neutralization of any of several peptides known to bind to E α^k E β^k , leads to retention of the C structure and a concomitant diminution in F or U dimers (Fig. 1a and b). For most E α^k E β^k to retain the C form, 50-100 μ M peptide is required; 10 μ M peptide has barely detectable effects (results not shown). Activity is related to binding to E α^k E β^k , as peptides lacking this property (hen egg lysozyme (HEL) residues 46-61, interacts with A α^k A β^k (ref. 10); ovalbumen

(OVA) residues 323–339, interacts with $\text{A}\alpha^d\text{A}\beta^d$ (refs 11, 12)) do not prevent loss of the C structure (Table 1).

We used western blotting to investigate high-affinity (slow dissociation rate) peptide binding. $\text{E}\alpha^k\text{E}\beta^k$ incubated at acid pH, then neutralized in the presence of biotinylated des-Ala-splice cytochrome peptide (DASP), remains associated with peptide throughout SDS-PAGE and western transfer. The strong peptide signal displayed at the C dimer position (Fig. 2a) correlates with efficient generation of the C form as determined by silver stain (Fig. 2b). Additional strong peptide signals at 90K and 110K, that have been previously observed, are present¹³; the chain composition of these forms is unknown, but they are distinct from F dimers (Fig. 2a, lane 4). $\text{E}\alpha^k\text{E}\beta^k$ incubated for 2 hours with peptide at neutral pH binds 17-fold less peptide, and no change in the amount of C dimer is detectable. Samples that are acid-treated then neutralized before DASP addition also show little peptide binding or C dimer (Fig. 2a). Therefore, generation of additional empty binding sites during low-pH treatment does not fully account for the marked enhancement of peptide binding observed in these experiments, as has been found using a functional assay¹⁴.

The apparent exchange of endogenous peptides for added DASP at pH 4.5, and the requirement for high (50–100 μM) peptide concentrations to maximally preserve the C state, are most consistent with an equilibrium reaction of low affinity (rapid dissociation rate) at acidic pH. However, differences between the pH required for dissociation of endogenous peptides^{15,16} versus binding of DASP could also explain these findings. To examine this issue, we pre-loaded $\text{E}\alpha^k\text{E}\beta^k$

molecules with biotinylated DASP, then exposed them to pH 4.5 for varying periods of time. Only 19% of pre-bound peptide remains bound to C-forms after incubation without additional peptide for 1.5 h at pH 4.5 and 37 °C (Fig. 3). Addition of 100 μM free biotinylated peptide 5 min before neutralization, results in a peptide signal similar to that of samples maintained at neutral pH. Compact dimers decrease from 85% to 15% of total class II if the preparation is exposed to low pH and neutralized in the absence of additional peptide, whereas C dimers totalling 44% of total protein are present if peptide is added. Longer (3.5 h) incubation at low pH leads to >95% loss of previously bound peptide and there is substantial binding of added peptide, which reaches 47% of the original level. A lower level of C molecules is achieved (13% without peptide, 27% with peptide at 3.5 h). Thus, enhanced dissociation and effective binding of the same peptide are both observed at pH 4.5. The interactions of peptide and class II required for promoting the C state occur rapidly at pH 4.5, with nearly half the maximum possible effect being seen within only 5 min of peptide-class II interaction. Further, the amount of C form and the extent of high-affinity peptide binding are directly proportional under these conditions.

The unusual kinetics of stable peptide binding to class II at neutral pH (refs 7, 17), the multiple discrete conformers of class II showing differential peptide association^{8,9,18}, the capacity of low pH to enhance peptide-class II interactions^{14,15}, and the role of acidic vesicles in antigen processing¹⁹, all led us to investigate whether peptide plays a substantial part in the folding state/stability of class II molecules achieved following transit out of an acidic environment. Our results provide direct evidence

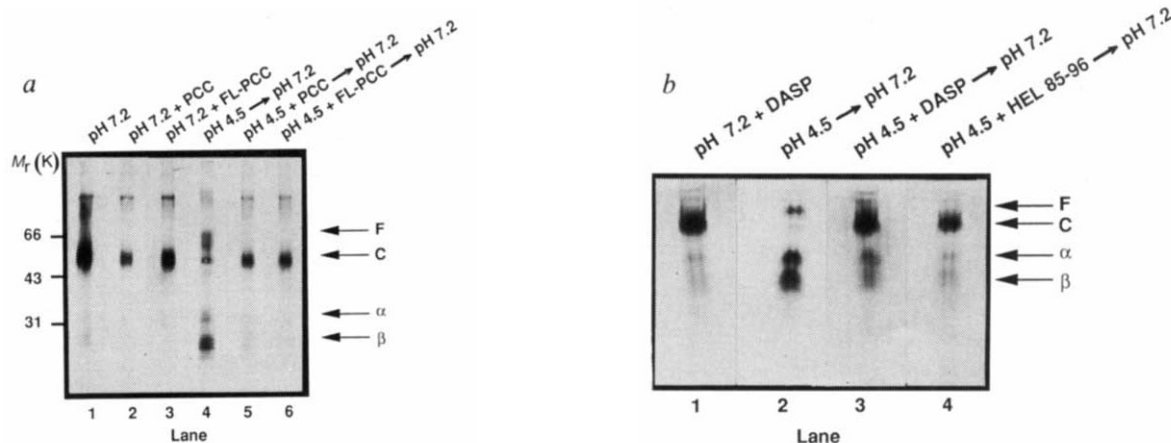


FIG. 1 The presence or absence of specific peptide determines the conformation of $\text{E}\alpha^k\text{E}\beta^k$ molecules leaving an acidic environment. **a**, Silver-stained SDS-polyacrylamide gel of $\text{E}\alpha^k\text{E}\beta^k$ in lipid vesicles: lane 1, incubated at pH 7.2 without added peptide; lane 2, incubated at pH 7.2 with pigeon cytochrome c (PCC) 88–104; lane 3, incubated at pH 7.2 with fluorescein-labelled PCC (FL-PCC) 88–104; lane 4, incubated at pH 4.5, then brought to pH 7.2 in the absence of peptide; lane 5, incubated at pH 4.5 with PCC 88–104, then brought to pH 7.2; lane 6, incubated at pH 4.5 with FL-PCC 88–104, then brought to pH 7.2. Numbers on the left of the figure represent molecular weight standards; F, floppy $\text{E}\alpha^k\text{E}\beta^k$ dimers (63–67K); C, compact stable $\text{E}\alpha^k\text{E}\beta^k$ dimers (56K); α , 33K α chain derived from U dimers dissociated in SDS buffer; and β , 27K β chain derived from U dimers dissociated in SDS buffer. Protein bands appear to be wide because of glycosylation of MHC and unreduced disulphide bonds present in MHC structure. **b**, Silver-stained SDS-polyacrylamide gel of $\text{E}\alpha^k\text{E}\beta^k$ in detergent micelles: lane 1, incubated at pH 7.2 with DASP; lane 2, incubated at pH 4.5, then brought to pH 7.2 without adding peptide; lane 3, incubated at pH 4.5 with DASP, then brought to pH 7.2; lane 4, incubated at pH 4.5 with HEL 85–96, then brought to pH 7.2. METHODS. $\text{E}\alpha^k\text{E}\beta^k$ was purified from lipopolysaccharide-stimulated CBA/J B cell blasts using monoclonal antibody 14-4-4 S (ref. 23) for affinity chromatography as described⁷. Purified $\text{E}\alpha^k\text{E}\beta^k$ was stored in 30 mM octylglucoside in Tris (100 mM), NaCl (140 mM), sodium azide (0.02%) (OG/Tris) at pH 8.3, 4 °C. For the experiment shown in Fig. 1a, the purified class II molecules were inserted in lipid vesicles as previously described⁷.

For the experiment shown in Fig. 1b, the purified class II molecules in OG/Tris were used directly. Aliquots of 50 μl of 1 μM $\text{E}\alpha^k\text{E}\beta^k$ at neutral pH (7.2) were either incubated at 37 °C without further additions or with a final concentration of 100 μM peptide. Identical aliquots were brought to pH 4.5 by adding 0.4 μl of 2.0 M acetic acid and incubated at 37 °C either without further additions or with 100 μM peptide added at the same time as the acid. After 30 min, the pH of the samples was readjusted to 7.2 by adding 0.4 μl of 1 M Tris, and the neutralized samples incubated for an additional 2–3 h at 37 °C. Later experiments have shown that this additional post-neutralization incubation is unnecessary for the effects reported here (see Fig. 3). Each sample (10 μl) was added to 25 μl sample buffer²⁴ without 2-mercaptoethanol, and loaded without prior boiling on a 12% linear polyacrylamide gel to permit assessment of the conformation/stability of the $\text{E}\alpha^k\text{E}\beta^k$ dimers. Gels were run at 200 V for ~45 min then silver stained²⁵. The effective pH for induction of unfolding and successful refolding was between 4.5–5, depending on such variables as whether $\text{E}\alpha^k\text{E}\beta^k$ was inserted into lipid vesicles or assayed in detergent micelles, and how soon after preparation the class II protein was tested. All peptides were prepared by solid phase synthesis and HPLC-purified before use. Sequence of PCC 88–104 is KAERADLIAYLKQATAK (one-letter amino-acid code); FL-PCC is PCC 88–104 with a fluorescein group on the amino terminus—this is a separate synthesis of the core PCC peptide; HEL 85–96, SSDITASVNCAL; DASP, KKANELIAYLKQATK.

FIG. 2 Peptide is most efficient in binding to $E\alpha^kE\beta^k$ and promoting the compact conformation if offered during low-pH transit. *a*, Western blot for bound peptide. Lane 1, $E\alpha^kE\beta^k$ incubated at pH 4.5 with biotinylated DASP (LCB-DASP), then brought to pH 7.2; lane 2, $E\alpha^kE\beta^k$ incubated at pH 4.5 without added peptide, brought to pH 7.2, then incubated with biotinylated DASP; lane 3, $E\alpha^kE\beta^k$ incubated at pH 7.2 with biotinylated DASP; lane 4, a lighter exposure of lane 1. *b*, Numbers are derived from a parallel silver-stained gel and represent the per cent of each conformation over total stained protein per lane.

METHODS. $E\alpha^kE\beta^k$ in OG/Tris was treated as described in the legend to Fig. 1 with the following changes: the peptide used was DASP conjugated on the amino terminus according to ref. 26. For detection of biotinylated peptide bound to $E\alpha^kE\beta^k$, MHC-peptide complexes were transferred from the SDS-polyacrylamide gel to nitrocellulose membranes (Hybond) for 1.5 h at 100 V in 20 mM Tris/150 mM glycine, pH 8.3. Biotinylated peptide associated with transferred proteins was detected with horseradish peroxidase-conjugated avidin and chemiluminescence (Enhanced Chemiluminescence Kit, Amersham). The protein distribution on silver-stained gels and the protein-bound biotinylated peptide signal revealed by chemiluminescence were measured by laser scanning densitometry using a Shimadzu CS-9000.

for an important positive contribution of peptide to the C structure of typical class II molecules, and indicate that this form is associated with the most stable peptide-binding state. Striking differences have been reported in the pH required for optimal binding of certain peptides versus that necessary for dissociation of complexes of the same peptide-class II pair already formed¹⁵. This is consistent with a significant effect of peptide on class II binding site conformation, an effect we find reflected in alterations in affinity for certain monoclonal anti-

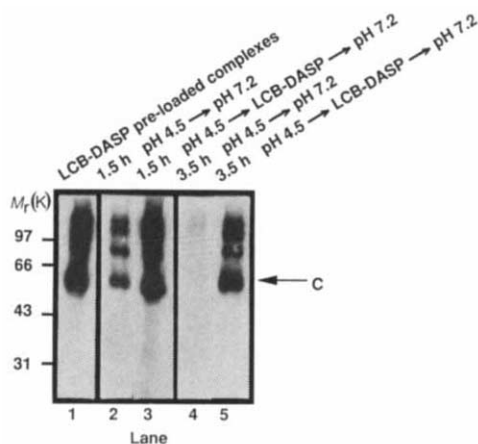
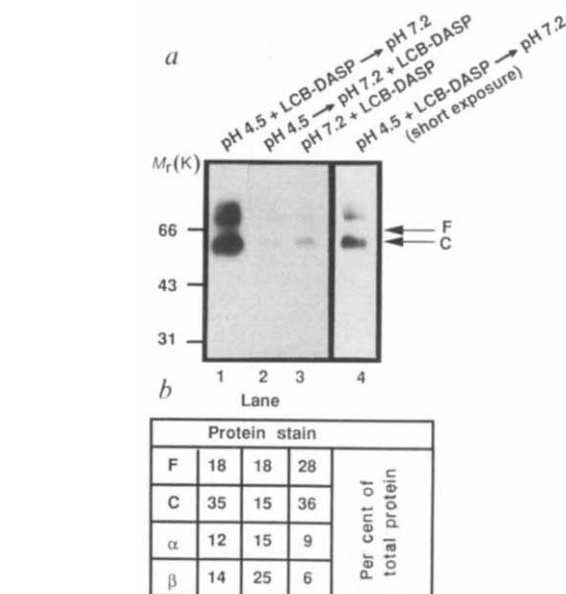


FIG. 3 Dissociation of biotinylated peptide from $E\alpha^kE\beta^k$ under acidic conditions and fast binding of peptide to empty molecules. Western blot showing bound peptide. Lane 1, $E\alpha^kE\beta^k$ molecules pre-loaded with biotinylated-DASP (LCB-DASP); lane 2, pre-loaded peptide: $E\alpha^kE\beta^k$ complexes incubated for 1.5 h at pH 4.5 without added peptide, then neutralized; lane 3, preloaded peptide: $E\alpha^kE\beta^k$ complexes incubated for 1.5 h at pH 4.5, incubated for 5 min with biotinylated DASP, then brought to pH 7.2; lane 4, same as lane 2, but incubated at pH 4.5 for 3.5 h; lane 5, same as lane 3, but incubated at pH 4.5 for 3.5 h before peptide addition.

METHODS. $E\alpha^kE\beta^k$: biotinylated DASP complexes were prepared by incubating 1 μ M $E\alpha^kE\beta^k$ with 100 μ M biotinylated DASP at neutral pH. $E\alpha^kE\beta^k$ containing a significant fraction of biotinylated DASP: $E\alpha^kE\beta^k$ complexes prepared in this manner were separated from free peptide using Millipore Ultrafree-MC filter units with a 10K cut-off point. This $E\alpha^kE\beta^k$ containing pre-bound biotinylated DASP was incubated at pH 4.5 and 37 °C for the times indicated and then neutralized either in the absence of additional peptide (lanes 2 and 4) or presence of 100 μ M biotinylated DASP peptide added 5 min before neutralization (lanes 3 and 5). Samples were immediately subjected to SDS-PAGE, one gel was then silver-stained, and a duplicate used for western blotting as described in the legend to Fig. 2.

bodies (S.S.N. and R.N.G., unpublished observations). Our observations of changes in class II structure during intracellular maturation and transport²⁰ are fully consistent with our *in vitro* data using purified class II free of invariant chain. These metabolic labelling studies showed an antigen-dependent conversion of U to C dimers in a post-Golgi compartment following invariant chain dissociation, the same change as observed here for acid-treated mature molecules stripped of endogenous peptide and re-exposed to exogenous peptide during neutralization.

It seems paradoxical that the same pH can enhance both dissociation of previously bound peptide and entry of added peptide into stable complexes. $E\alpha^kE\beta^k$ forms short-lived but specific peptide complexes at neutral pH that slowly convert to long-lived forms associated with the C state⁷. These data may indicate a rigidity of class II at neutral pH that only rarely permits conformational change of the binding region, retarding both formation and dissociation of C complexes. At low pH, the class II molecule may gain more flexibility, facilitating exit from the C state and loss of previously bound peptide. Added peptide could then enter available sites and efficiently drive the system towards, if not fully into, the C state. Additional class II stabilization upon emergence from the acidic environment would then give rise to typical long-lived complexes. This model, similar to one already proposed²¹, implies that peptide acquisition by class II in an acid compartment, followed by emergence into a neutral environment, results in the 'trapping' of processed antigen as stable complexes. This may contribute to the ability of class II to present peptides with a low effective affinity in the acidic environment where initial interaction may take place. The apparent absence of a similar stabilizing mechanism for class I-peptide complexes may limit this limb of the antigen presentation system to peptides with a high intrinsic affinity for class I, perhaps explaining observations suggesting a smaller useful number of determinants in various proteins for class I versus class II-dependent T-cell stimulation²². □

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Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases

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MITOGEN activated protein (MAP) kinases (MAPKs) are a family of protein-serine/threonine kinases activated as an early intracellular response to a variety of hormones and growth factors^{1–4}. They are unique in requiring both serine/threonine and tyrosine phosphorylation to become active⁵ and are the only examples of protein-serine/threonine kinases activated by tyrosine phosphorylation. Nerve growth factor (NGF) promotes differentiation of phaeochromocytoma (PC12) cells, which respond by conversion within hours from a chromaffin-like to a sympathetic neuron-like phenotype^{6,7}. NGF stimulation of PC12 cells increases the activity of two protein kinases by >20-fold within minutes⁸, both strikingly similar to MAPKs. They are inactivated by either protein-tyrosine phosphatases or the protein-serine/threonine phosphatase termed protein phosphatase 2A (ref. 8), they activate protein S6 kinase-II (refs 9, 10), and they phosphorylate identical threonine residues on myelin basic protein (our unpublished results) to those phosphorylated by other MAPKs^{11,12}. Immunological data¹³ indicate that these protein kinases, termed peak-I and peak-II (Fig. 1a) are probably ERK2 and ERK1, respectively, two widely expressed MAPK isoforms¹³. Here we identify the 'MAP kinase kinases' (MAPKKs) in PC12 cells which are activated by NGF and report that MAPKKs are dependent on serine/threonine phosphorylation for activity and promote phosphorylation of serine/threonine and tyrosine residues on MAPKs.

We screened the fractions from Mono Q in Fig. 1a for activities able to reactivate the peak-I MAPK previously inactivated by incubation with either CD45 (a protein-tyrosine phosphatase (PTPase)) or protein phosphatase 2A (PP2A). These experiments revealed Mg-ATP-dependent reactivating activity using either substrate, that was confined to fractions eluting just before and overlapping with the peak-I MAPK (Fig. 1a) and which is hereafter referred to as MAPKK. MAPKK activity was pooled (Fig. 1a) and subjected to successive chromatographies on Mono S, Mono Q and Superose 12 (Fig. 1). Identical profiles were obtained whether MAPKK was assayed with CD45-treated MAPK ('MAP(Tyr)KK') or PP2A-treated MAPK ('MAP(Ser/Thr)KK'). MAPKK was resolved into two peaks on Mono Q (Fig. 1c), both possessing the same MAP(Tyr)KK: MAP(Ser/Thr)KK activity ratio. Each eluted

from Superose as proteins of relative molecular mass 50,000–55,000 (M_r 50–55K) (Fig. 1d). If NaCl was omitted, MAP(Tyr)KK and MAP(Ser/Thr)KK again coeluted, but with an apparent M_r of 40K (not shown).

Extracts from unstimulated cells contain inactive MAPK, which elutes from Mono Q at the position of active peak-I MAPK (Fig. 2a). Inactive MAPK was reactivated by purified MAPKK using [γ -³²P]ATP, and rechromatography resolved two active components (Fig. 2c) coeluting with the peak-I and peak-II MAPKs. Thus inactive MAPK in unstimulated cells (Fig. 2a) is the precursor of both active MAPK isoforms, and MAPKK can activate each species. The ³²P-labelled MAPKs migrated as 42K (peak-I) and 44K (peak-II) proteins (Fig. 2c inset), consistent with their identity as ERK2 and ERK1, respectively¹³. Both the peak I (Fig. 2d) and peak II (not shown) isoforms were phosphorylated on serine/threonine and tyrosine, establishing that MAPKK phosphorylates both types of phosphoamino acid. Incubation with PTPases inactivated peak I and II by >90%, released 30% of the ³²P radioactivity (not shown) and dephosphorylated tyrosine specifically (Fig. 2d), whereas incubation with PP2A also inactivated peaks I and II by >90%, released 70% of the ³²P radioactivity (not shown), and dephosphorylated serine and threonine specifically (Fig. 2d). No MAPKK activity was detectable when extracts from unstimulated cells were chromatographed on Mono Q (Fig. 2b), demonstrating that activation of MAPKK requires NGF stimulation.

MAP(Tyr)KK and MAP(Ser/Thr)KK were both inactivated by preincubation with PP2A (Fig. 3a, b). Inactivation was blocked by the PP2A inhibitor okadaic acid¹⁴, but unaffected by orthovanadate (0.2 mM), a general PTPase inhibitor, indicating that PP2A did not activate contaminating PTPase which then inactivated MAPKK. Consistent with dephosphorylation of serine/threonine residues, three PTPases (CD45 (ref. 15), LAR (ref. 16) and T-Cell phosphatase (ref. 17)) had no effect on either MAP(Tyr)KK or MAP(Ser/Thr)KK (Fig. 3a, b) under conditions where they inactivated MAPK by 80% (not shown). Although PP2A dephosphorylates some tyrosine residues in the presence of divalent cations¹⁸, inactivation of MAPKK by PP2A does not seem to involve tyrosine dephosphorylation because inactivation occurs without divalent cations. In addition preincubating PP2A with 0.2 mM pyrophosphate or 1 mM ATP (which inactivates its protein serine/threonine phosphatase activity while enhancing its PTPase activity¹⁸) prevents inactivation of MAPKK (not shown).

MAPK inactivated by either CD45 or PP2A shows no reactivation on subsequent incubation with Mg-ATP, demonstrating that neither the tyrosine nor the serine/threonine residues are phosphorylated at significant rates by MAPK itself, and suggesting that MAPKK is a protein kinase. But an interaction may occur between MAPKK and MAPK inducing conformational changes that enhance enormously the rate of autophosphorylation of MAPK. This idea was raised by Ahn *et al.*¹⁹, who recently identified two MAPK activators in epidermal growth factor (EGF)-stimulated 3T3 cells. These resemble our MAPKKs because they reactivate MAPK inactivated by either CD45 or PP2A (ref. 19).

MAPK is activated by tyrosine phosphorylation and stimulated by NGF and other growth factors whose receptors are protein-tyrosine kinases^{1,2,20}. Consequently, there had been optimism that very few intervening steps might exist between receptor and MAPK activation. This hope is dashed by our results, which indicate that MAPKK is activated by serine/threonine phosphorylation, implying that 'MAP kinase kinase' is a protein-serine/threonine kinase (Fig. 4). But one can now propose how okadaic acid and phorbol esters, which inhibit protein-serine/threonine phosphatases and activate protein kinase C (a serine/threonine kinase), respectively, might cause tyrosine phosphorylation and activation of MAPKs (refs 2, 21). These tumour promoters may stimulate serine/threonine phosphorylation of MAPKK.

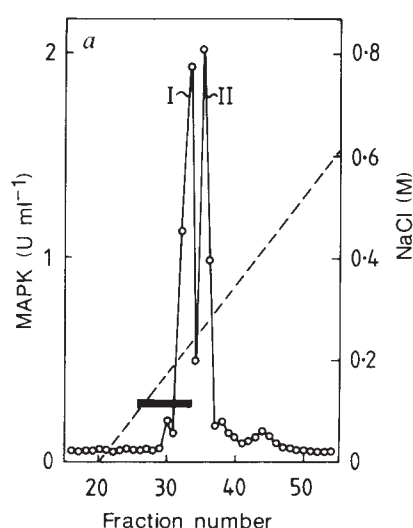
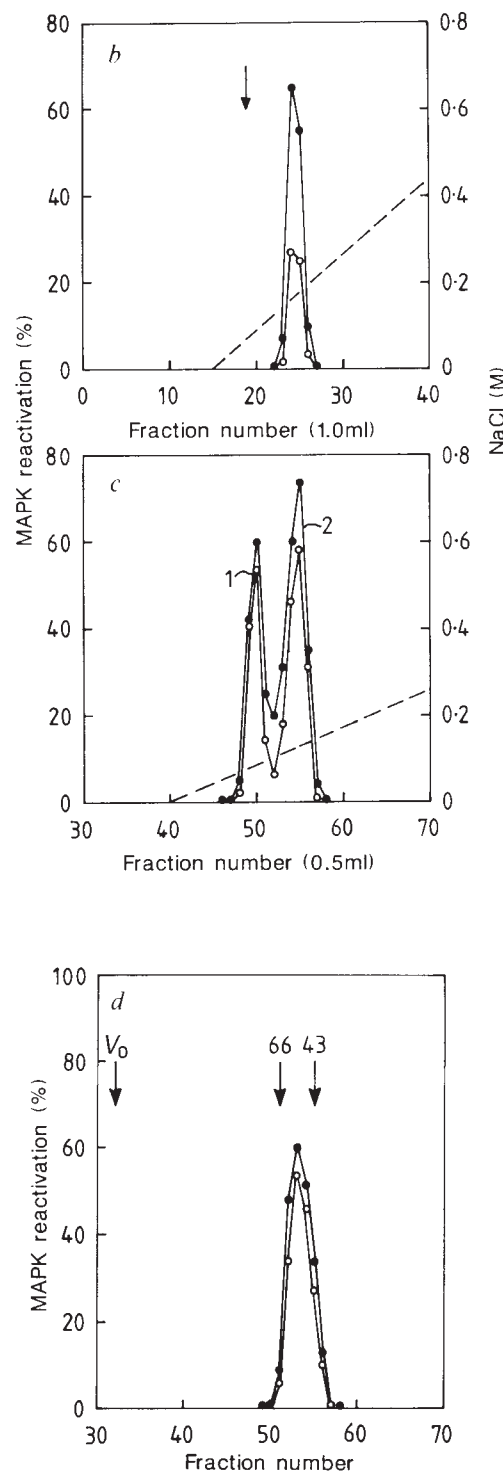


FIG. 1 Coelution of MAP(Tyr)KK and MAP(Ser/Thr)KK. *a*, PC12 cells were stimulated for 15 min with 50 ng ml^{-1} NGF, lysed, and the extracts from 10 dishes (4 ml, 6×10^7 cells) diluted to 10 ml in 50 mM Tris-HCl pH 7.3 (20 °C), 1.5 mM EGTA, 0.1% (v/v) 2-mercaptoethanol (buffer A), containing 0.15 mM sodium orthovanadate, 1 mM benzamidinium and $4 \mu\text{g ml}^{-1}$ leupeptin and applied to a $5 \times 0.5 \text{ cm}$ column of Mono Q (Pharmacia) equilibrated in the same buffer. After washing with 10 ml buffer, the column was developed with a 40 ml linear salt gradient (---) from 0 to 0.7 M NaCl at a flow rate of 1 ml min^{-1} . Fractions of 1.0 ml were collected and assayed for MAPK activity (○). Similar results were obtained in more than 15 experiments. The fractions containing MAPKK activity are shown by a horizontal bar and were located as described in methods. No MAPKK activity was detectable in any other fraction in three experiments using different preparations of NGF-stimulated PC12 cells. *b*, Chromatography on Mono S. The fractions from Mono Q (Fig. 1*a*) containing MAPKK activity were pooled, dialysed against 25 mM 2-(*N*-morpholino) ethanesulphonic acid (MES) buffer pH 6.5 containing 0.2 mM EGTA, 0.03% (w/v) Brij 35, 5% (v/v) glycerol, 1 mM benzamidinium, $4 \mu\text{g ml}^{-1}$ leupeptin and 0.1% (v/v) 2-mercaptoethanol, and chromatographed on a $5 \times 0.5 \text{ cm}$ column of Mono S (Pharmacia) equilibrated in the same buffer. After sample application, the column was developed with a linear salt gradient (---) to 0.7 M NaCl. The flow rate was 1.0 ml min^{-1} and fractions of 1.0 ml were collected and assayed for MAP(Tyr)KK (●) and MAP(Ser/Thr)KK (○) as described in methods. The arrow denotes the elution position of the peak-I isoform of MAPK, which was pooled with the fractions from Mono Q (*a*). Similar results were obtained in three different experiments. *c*, Chromatography on Mono Q. The active fractions from Mono S (Fig. 1*b*) were pooled, dialysed against 50 mM Tris-HCl pH 7.3, 2 mM EGTA, 2 mM EDTA, 0.1% (v/v) 2-mercaptoethanol, 5% (v/v) glycerol, 0.03% Brij 35, 1 mM benzamidinium and $4 \mu\text{g ml}^{-1}$ leupeptin, and chromatographed on Mono Q ($5 \times 0.5 \text{ cm}$) equilibrated in the same buffer. After sample application, the column was developed with a 40 ml linear salt gradient (---) to 0.7 M NaCl. The flow rate was 1.0 ml min^{-1} and fractions of 0.5 ml were collected and assayed for MAP(Tyr)KK (●) and MAP(Ser/Thr)KK (○) activities. *d*, The peak-2 MAPKK from Mono Q in *c* was concentrated to 0.2 ml by centrifugal ultrafiltration, and applied to a $30 \times 1.5 \text{ cm}$ column of Superose 12 (Pharmacia) equilibrated in buffer A containing 0.2 M NaCl, 1 mM benzamidinium, $4 \mu\text{g ml}^{-1}$ leupeptin and 0.03% Brij 35. The flow rate was 0.3 ml min^{-1} and fractions of 0.25 ml were collected. Aliquots ($5 \mu\text{l}$) were assayed for MAP(Tyr)KK (●) and MAP(Ser/Thr)KK (○) for 20 min as described in methods. The arrows denote the elution positions of the void volume (V_0) and the marker proteins bovine serum albumin (66K) and ovalbumin (43K). No MAPKK activity was detected in any other fraction in several different experiments.

METHODS. PC12 cells were cultured, stimulated with NGF and lysed as described previously⁸, except that the lysis buffer was 20 mM Tris-acetate pH 7.0 (20 °C), 0.27 M sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate, 10 mM sodium β -glycerophosphate, 50 mM NaF, 5 mM sodium pyrophosphate, 1% Triton X-100, 1 mM benzamidinium, $4 \mu\text{g ml}^{-1}$ leupeptin, 0.1% (v/v) 2-mercaptoethanol. MAPK was assayed as described⁸ and one unit of activity (U) is that amount which catalyses the incorporation of 1.0 nmol phosphate into myelin basic protein in 1 min. To assay MAPKK, the peak-I isoform of MAPK (*a*) was bound to phenyl-Superose in the presence of 0.25 M NaCl and eluted with 50% ethylene glycol¹, a procedure that separated it from MAPKK which is not retained at 0.25 M NaCl. The



MAPK was then dialysed against 50 mM Tris-HCl pH 7.0 (20 °C), 1.5 mM EGTA, 0.03% Brij 35, 0.1% (v/v) 2-mercaptoethanol, 5% (v/v) glycerol, 1 mM benzamidinium, $4 \mu\text{g ml}^{-1}$ leupeptin. To assay MAP(Ser/Thr)KK, MAPK (0.3 ml , 1.0 U ml^{-1}) was inactivated by 90% by incubation for 30 min at 30 °C with 0.3 ml of PP2A catalytic subunit (60 mU ml^{-1} in buffer B; see ref. 26 for preparation and definition of units) and 0.3 ml of $10 \mu\text{M}$ okadaic acid then added to inactivate PP2A. Aliquots ($15 \mu\text{l}$) were mixed with column eluate ($5 \mu\text{l}$) and then assayed for MAPKK as described in the legend to Fig. 2. MAP(Tyr)KK was assayed in the same way except that MAPK was inactivated by 90% by incubation for 45 min with a PTPase (CD45, $32 \mu\text{g ml}^{-1}$ in buffer B) rather than PP2A, and 2 mM sodium orthovanadate (a protein-tyrosine phosphatase inhibitor) replaced okadaic acid. MAP(Tyr)KK and MAP(Ser/Thr)KK activities are plotted as per cent reactivation of MAPK in the assays, where 100% corresponds to the level of MAPK activity before its inactivation with PP2A or protein-tyrosine phosphatases.

MAP(Tyr)KK and MAP(Ser/Thr)KK coelute on anion and cation exchange resins, gel filtration at different ionic strengths (Fig. 1), and are inactivated in parallel by PP2A (Fig. 3), indicating they are functions of a single enzyme (complex). Several protein kinases (of unknown function) have been identified that autophosphorylate on tyrosine and serine/threonine residues²²⁻²⁵. Whether these are related to MAPKKs requires further investigation.

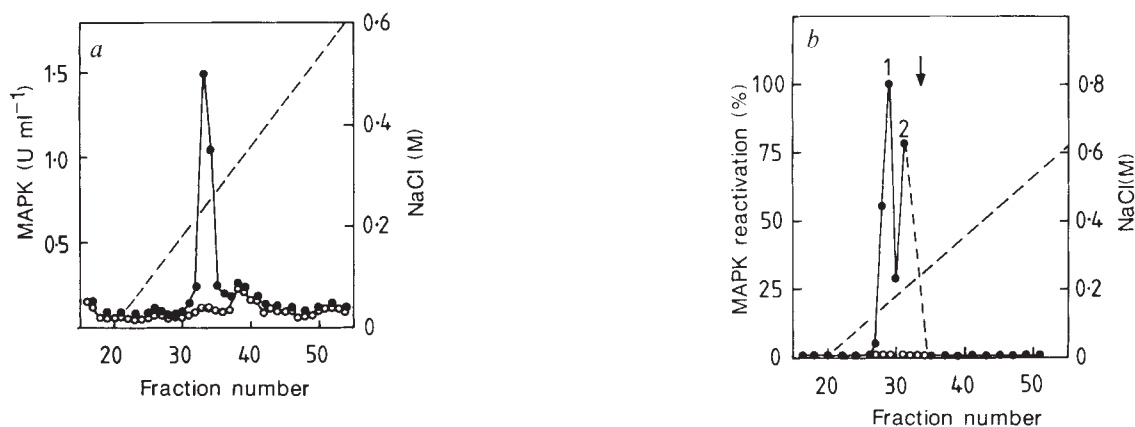
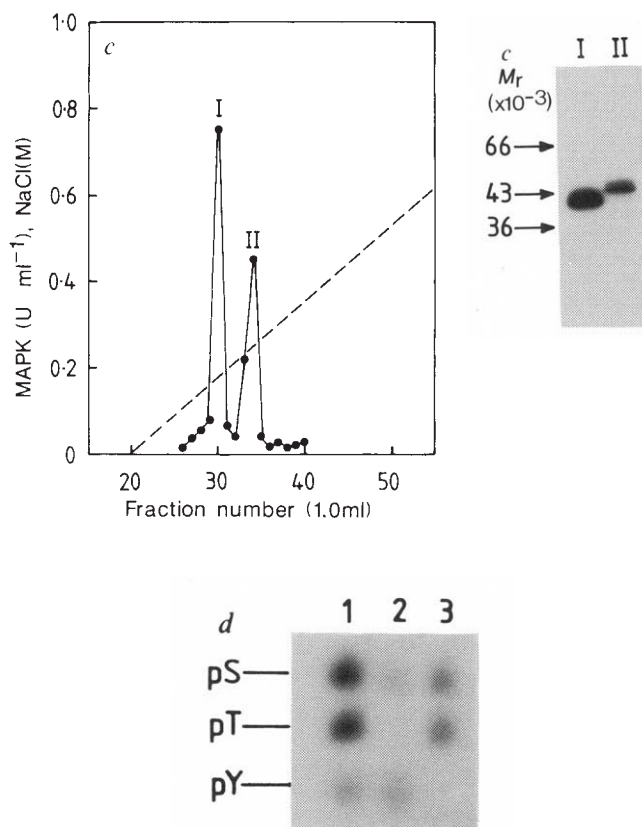


FIG. 2 Activation and inactivation of MAPKs by MAPKK and protein phosphatases. *a*, Lysates from 6×10^7 unstimulated PC12 cells (4 ml) were chromatographed on Mono Q as described in Fig. 1a and assayed for MAPK without any preincubation ($\circ$), or after incubation with Mg-ATP and MAPKK from NGF-stimulated cells (fraction 29 from *b*) ($\bullet$). Incubation with Mg-ATP alone did not produce any activation. Similar results were obtained in six experiments with different batches of cells. *b*, Mono Q fractions from the NGF-stimulated cells in Fig. 1a ($\bullet$) and the unstimulated cells in Fig. 2a ($\circ$) were tested for their ability to stimulate inactive MAPK (fraction 33 in *a*) using the assay described in methods. The value of 100% is the activation achieved in *a* by fraction 29 in this figure. The peak-I isoform of MAPK overlaps with the peak-2 form of MAPKK making it difficult to assay the second half of the latter peak accurately, which is therefore shown by a broken line. Similar results were obtained in six separate chromatographies. *c*, Inactive MAPK from control cells (*a*) was activated by the purified peak-2 form of MAPKK (Fig. 1c) using [γ - 32 P]ATP (2×10^6 c.p.m. nmol^{-1}), and the solution passed through Sephadex G25 (to remove ATP) and chromatographed on phenyl-Superose to remove MAPKK and further purify MAPK (see legend to Fig. 1). MAPK was eluted from phenyl-Superose with 50% (v/v) ethylene glycol, diluted 10-fold into Mono Q equilibration buffer and chromatographed on Mono Q as in Fig. 1c. The positions of the peak-I and peak-II isoforms of MAPK ($\bullet$) are marked. Inset, the 32 P-labelled peak-I (I) and peak-II (II) MAPKs were concentrated by centrifugal ultrafiltration, subjected to SDS-PAGE²⁷ and autoradiographed. The arrows denote the elution of the marker proteins bovine serum albumin (66K), ovalbumin (43K) and glyceraldehyde 3-phosphate dehydrogenase (36K). *d*, Phosphoamino acid analysis of 32 P-labelled peak-I MAPK after incubation for 60 min at 30 °C in the absence of protein phosphatases (lane 1), with 30 mM ml^{-1} PP2A (lane 2) or with 125 μg ml^{-1} LAR (lane 3). MAPK was inactivated by >90% after treatment with either phosphatase. The positions of phosphoserine (pS), phosphothreonine (pT) and phosphotyrosine (pY) are marked. Very similar results were obtained using the 32 P-labelled peak-II MAP kinase from *c* (not shown) or when LAR was replaced by CD45 (not shown). A MAPK from 3T3 cells (which appears to be ERK2 (ref. 28)) was reported to be phosphorylated predominantly on threonine and tyrosine *in vivo*³ and these residues have been shown to occur in a thr(P)-glu-tyr(P) sequence²⁹ that is also present in ERK1 (ref. 13) and located close to the putative ATP-binding site. The phosphorylation of serine, in addition to threonine and tyrosine, by MAPKK may reflect an *in vitro* phosphorylation not observed *in vivo*, or contamination of partially purified MAPKK with another protein kinase that phosphorylates serine residues on MAPK. But the level of phosphoserine detected is critically dependent on the time of hydrolysis, as this phosphoamino acid is much more acid-labile than the phosphothreonine.

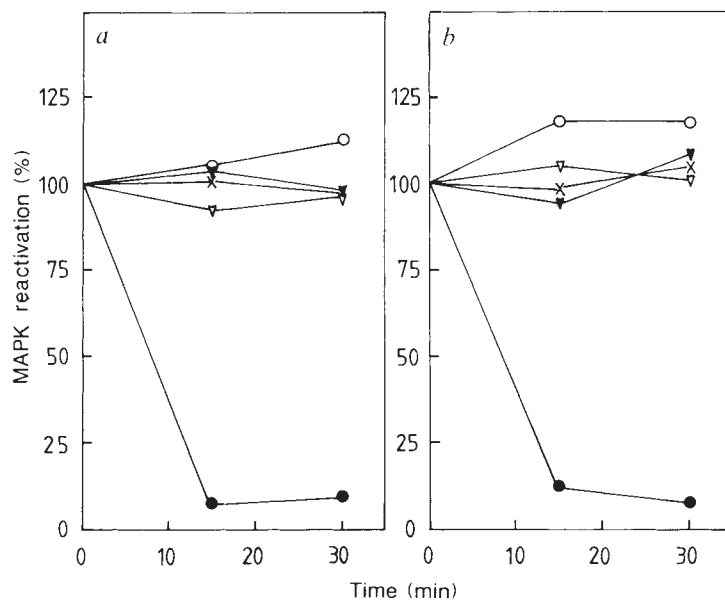
METHODS. MAPKK in *b* was assayed at 30 °C using inactive MAPK (fraction 33 from *a*). Inactive MAPK (5 μl) was mixed with 10 μl 50 mM Tris-HCl pH 7.0 (20 °C), 0.1 mM EGTA, 0.1% (v/v) 2-mercaptoethanol and 1 mg ml^{-1} BSA (buffer B) and 5 μl of Mono Q eluate. After incubation for 3 min at 30 °C the MAPKK reaction was initiated with 10 μl of 50 mM magnesium acetate/0.3 mM ATP. After a further 20 min at 30 °C, 20 μl of a solution

Why are MAPKs activated by combined serine/threonine and tyrosine phosphorylation, even though tyrosine phosphorylation does not provide a direct link to receptor tyrosine kinases? Furthermore, phosphorylation of serine/threonine and tyrosine by one enzyme implies that integration of signals from two different pathways is not required for activation of MAPK as suggested in ref. 5. This unique mechanism of activation may impart exquisite specificity for activation by MAPKK,



containing 62.5 mM Tris-HCl pH 7.0 (20 °C), 0.83 mg ml^{-1} myelin basic protein, 2.5 μM PKI (the specific 20-residue peptide inhibitor of cyclic AMP-dependent protein kinase³⁰) and 0.25 mM [γ - 32 P]ATP (10^6 c.p.m. nmol^{-1}) was added. Incorporation of phosphate into myelin basic protein was measured 10 min later as described⁸. The 32 P-labelled MAPK from *c* was precipitated with 25% (w/v) trichloroacetic acid. After centrifugation for 5 min at 13,000g, the pellets were washed three times with ether to remove trichloroacetic acid, dried and hydrolysed for 1.5 h at 110 °C with 6 M HCl and redried. Finally, the samples were dissolved in 2 μl of pyridine acetate pH 3.5 (water:acetic acid:pyridine, 945:50:5), subjected to electrophoresis on thin layer cellulose (Kodak) and autoradiographed.

FIG. 3 MAPKK is inactivated by PP2A, but not by protein-tyrosine phosphatases. All incubations were done at 30 °C. MAPKK from Mono S (Fig. 1b) was dialysed against 25 mM MES buffer pH 6.5 containing 0.2 mM EGTA, 0.03% Brij 35, 5% (v/v) glycerol, 1 mM benzamidine and 4 $\mu\text{g ml}^{-1}$ leupeptin and a 5 μl aliquot incubated for the times indicated with 2.5 μl of PP2A in buffer B, and the reactions terminated by addition of 2.5 μl 10 μM okadaic acid. MAPKK was incubated with PTPases in an identical manner, except that 5 mM EDTA was present and the reactions were terminated with 2 mM sodium orthovanadate. MAPKK was then tested for its ability to reactivate the peak-I MAPK that had been inactivated by incubation with either PP2A (MAP(Ser/Thr)KK) or PTPases (MAP(Tyr)KK). MAPK (90 μl , 1.0 U ml^{-1}) was incubated for 30 min with 45 μl PP2A (90 mU ml^{-1} in buffer B) or for 45 min with 45 μl CD45 (48 $\mu\text{g ml}^{-1}$ in buffer B) and the reactions stopped with 45 μl 10 μM okadaic acid (PP2A) or 2 mM sodium orthovanadate (CD45). Aliquots of inactivated MAPK (10 μl) were then mixed with MAPKK (10 μl) that had been preincubated with different phosphatases, and MAPKK reactions initiated with 10 μl 50 mM magnesium acetate/0.3 mM ATP. After incubation for 20 min, MAPK activity was assayed for 10 min as described in Fig. 2. Control incubations carried out in the absence of MAPKK showed no reactivation. MAP(Tyr)KK (a) or MAP(Ser/Thr)KK activity (b) are plotted as per cent reactivation of MAPK, where 100% is the activity of MAPK inactivated with PP2A or CD45 and reactivated with MAPKK that had not been treated with any phosphatase and stored on ice. MAPKK was preincubated with 30 mU ml^{-1} PP2A (●), 30 mU ml^{-1} PP2A + 10 μM okadaic acid (○), 16 $\mu\text{g ml}^{-1}$ CD45 (×), 125 $\mu\text{g ml}^{-1}$ LAR (▼), or 75 $\mu\text{g ml}^{-1}$ T-cell phosphatase (▽). Similar results



were obtained with four preparations of MAPKK at different states of purity. The peak-1 and peak-2 MAPKKs were affected by phosphatases in a similar manner. PP1(30 mU ml^{-1}), a more specific okadaic acid-sensitive protein-serine/threonine phosphatase had no effect on either peak 1 or peak 2 MAPKK activity (not shown).

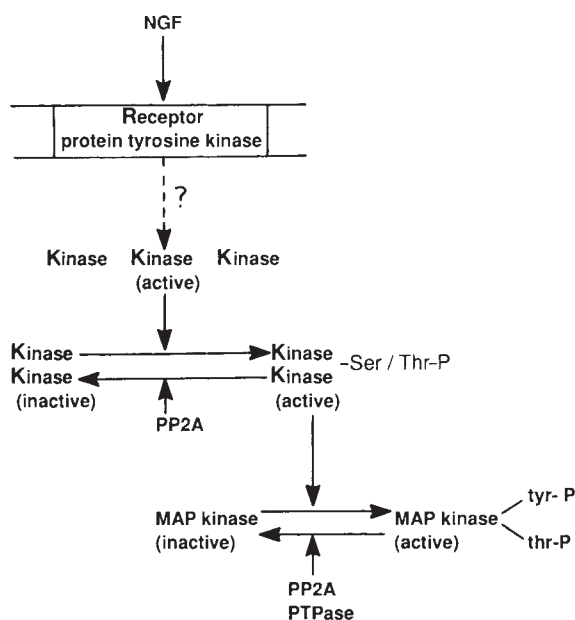


FIG. 4 The protein kinase cascade by which nerve growth factor activates MAPK. The NGF receptor involved in cell signalling is the *trk* proto-oncogene, a protein-tyrosine kinase²⁰. Interaction of NGF with *trk* activates the associated protein-tyrosine kinase, which triggers activation of the MAPK cascade by an unknown mechanism. If MAPKK is inactivated with PP2A (and okadaic acid then added to inhibit PP2A), subsequent preincubation with Mg-ATP does not lead to reactivation, suggesting that a distinct MAP kinase kinase kinase is required to reactivate MAPKK. The data indicate that MAP kinase kinase kinase is a protein-serine/threonine kinase that activates MAPKK allowing the latter to activate MAPK by promoting combined serine/threonine and tyrosine phosphorylation. The major physiological targets of MAPK are likely to be other protein-serine/threonine kinases^{9,10}. MAPK is inactivated by either PP2A or PTPases.

preventing activation of MAPKs by the myriad of other cellular protein kinases. Alternatively, activation by tyrosine phosphorylation may permit inactivation of MAPK by a specific PTPase that is regulated by as yet unidentified mechanisms. □

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Isolation of the human *cdk2* gene that encodes the cyclin A- and adenovirus E1A-associated p33 kinase

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CYCLINS are regulatory subunits which associate with kinases to form complexes that control many of the important steps in cell-cycle progression. The best characterized of the cyclin-containing complexes is the association of cyclin B with the p34^{cdc2} kinase. The p34^{cdc2}/cyclin B complex is required for the G2 to M transition (see refs 1–4 for review), but the physiological role of other cyclin complexes is unclear. Human cyclin A binds independently to two kinases, associating with either p34^{cdc2} or a related protein, p33 (refs 5–7). In adenovirus-transformed cells, the viral E1A oncoprotein seems to associate with p33/cyclin A but not with p34^{cdc2}/cyclin A (B. Faha, M.M., L.H.T. and E.H., manuscript submitted). To isolate the gene for p33, we have cloned several novel human *cdc2*-related genes. The protein product of one of these genes, *cdk2* (cyclin-dependent kinase 2), shares 65% sequence identity with p34^{cdc2} (ref. 8) and 89% identity with the *Xenopus Eg-1* gene product⁹. Immunochemical characterization and partial proteolytic mapping show that the *cdk2* gene product is the cyclin A-associated p33. Immunoprecipitations of the p33^{cdk2} protein suggest that it can act as a protein kinase *in vitro*. As p33^{cdk2} is bound to cyclin A and is targeted by the viral E1A protein, we suggest that the p33^{cdk2}/cyclin A complex has a unique role in cell-cycle regulation of vertebrate cells.

Several antibodies specific for human cyclin A have been used to characterize associated proteins^{5–7}. One of these proteins, of relative molecular mass 34,000 (*M_r*, 34K), is a polypeptide that has been identified as human p34^{cdc2} (B. Faha, M.M., L.H.T. and E.H., manuscript submitted). A second cyclin A-associated protein with a *M_r* ~ 33K (p33) has also been characterized^{6,7}. Several lines of evidence suggest that p33 and p34^{cdc2} are related but distinct polypeptides. First, p33 migrates slightly faster than the fastest-migrating form of p34^{cdc2} on SDS-polyacrylamide gels (refs 5, 6 and Fig. 1a). Second, when recovered from cyclin A immunoprecipitations, p33 is recognized by antiserum⁸ against a peptide derived from the highly conserved PSTAIRE region of *cdc2* proteins⁶; however, p33 is not recognized by anti-peptide antibodies¹⁰ raised against the carboxy-terminal region of p34^{cdc2} (ref. 6 and B. Faha *et al.*, manuscript submitted). Third, on two-dimensional isoelectric focusing gels, p33 resolves into two species that are distinct from p34^{cdc2} (ref. 7). Furthermore, the *Staphylococcus aureus* V8 partial proteolytic map of p33 is different from that of p34^{cdc2}, although the N-chloro-succinimide digestion patterns of p33 and p34^{cdc2} are very similar⁷. Together, these observations suggest that p33 shares some properties with p34^{cdc2}, even though they are clearly distinct proteins. The p33 protein can be further distinguished from p34^{cdc2} by its association with adenovirus E1A. Our laboratory has previously shown that adenovirus E1A is associated with a 60K polypeptide¹¹ that has since been identified as human cyclin A (refs 5, 6). E1A immunoprecipitations contain the p33 protein in addition to cyclin A but do not have any detectable p34^{cdc2} (ref. 7 and B. Faha *et al.*, manuscript submitted). The association of cyclin A with p33 and p34^{cdc2} is illustrated in Fig. 1a. Lane 3 shows that p34^{cdc2} and p33 were coprecipitated with a monoclonal antibody specific for cyclin A, and lane 2 shows that only p33 was coprecipitated with E1A.

On the basis of its properties, we hypothesized that p33 might be encoded by a *cdc2*-related gene. To test this hypothesis, we

set out to clone *cdc2*-related genes. Degenerate oligonucleotides derived from conserved regions of the *cdc2* gene family¹² were used to amplify complementary DNA made from two cell lines, human cervical carcinoma (HeLa) and human pre-B leukaemia (Nalm-6) cells, using the polymerase chain reaction¹³ (PCR). The PCR fragments were cloned into plasmids, sequenced and used to screen cDNA libraries for full-length clones. Eight novel human *cdc2*-related genes have been obtained so far. The production and characterization of these clones will be described in more detail elsewhere.

One of these clones contained an open reading frame encoding a protein of 298 amino acids (Fig. 1b). The initial sequencing of this clone showed that it had several of the properties that we expected for p33. The predicted amino-acid sequence from this clone showed 65% identity with human *cdc2* (ref. 8) and had the hallmarks of a protein kinase¹⁴. Of note are the sequence GXGXXG (residues 11–16) found in nucleotide-binding proteins, the invariant lysine residue involved in the phosphotransfer reaction (residue 33), and the APE motif (residues 170–172) found in protein kinase catalytic domains. The sequence also closely resembled that of a known *cdc2*-like kinase, the *Xenopus Eg-1* gene product, showing 89% identity at the amino-acid level⁹ (Fig. 1b). Recently a new convention for naming these *cdc2*-like kinases has been established. If the kinase binds to a cyclin, it is classified as a cyclin-dependent kinase (cdk). Numbers are assigned on the basis of discovery date. Therefore the *Xenopus Eg-1* gene can also be described as *Xenopus cdk2*. Because of the close homology to the *Xenopus cdk2*, we assume that the cDNA clone in Fig. 1b is human *cdk2*.

The coding region of human *cdk2* has a number of characteristics that link it closely to the *cdc2* family. Its sequence is identical

FIG. 1 a, Presence of a 33K protein in cyclin A and E1A immunoprecipitations. Lysates of the adenovirus-transformed human 293 cell line²⁶ were immunoprecipitated with: PAb416, a monoclonal antibody against simian virus 40 large-T antigen²⁷ used as a negative control (lane 1); M73, a monoclonal antibody against E1A protein²⁸ (lane 2); C160, a monoclonal antibody against human cyclin A protein^{5,29} (lane 3); normal rabbit serum (lane 4); and 114 (LTI/BRL-Gibco: now referred to as anti-p34^{cdc2} kinase peptide antibody 3398 SA), a commercial polyclonal rabbit serum raised against a peptide derived from the C terminus of p34^{cdc2} (lane 5). Positions of cyclin A, p33 and p34^{cdc2} are indicated. b, *cdk2* cDNA sequence and deduced amino-acid sequence (single-letter amino-acid code). Bold letters indicate the conserved region containing the PSTAIRE motif and the underlined sequences indicate the regions that were selected for the design of the *cdc2*-kinase-family PCR primers. The deduced amino-acid sequence of the *Xenopus Eg-1/cdk2* gene is shown below for comparison; dashes represent residues identical between human and *Xenopus cdk2*.

METHODS. a, Semi-confluent monolayers of 293 cells were labelled with 400 μ Ci [³⁵S]methionine (NEN Express) per dish in methionine-free medium for 4 h, and lysed in buffer containing 50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 5 mM EDTA, pH 8.0, 0.1% Nonidet P-40, 50 mM NaF and 1 mM PMSF. Immunoprecipitations were as described²⁸. b, The sequences of the primers used for enzymatic amplification¹³ of human *cdc2*-family kinases were: primer 1, 5'-AGTTCATGAATTCTGAGAAGAT(CT)GG(ACGT)GAGGG(ACGT)-AC(ACGT)TA-3'; primer 2, 5'-TAAGATCCGAATTCACCTCGGG(ACGT)G(AC)- (ACGT)CG(AG)TACCA. For the isolation of template DNA, first-strand cDNA was prepared from total cytoplasmic RNA made from HeLa cervical carcinoma cells or Nalm-6 pre-B leukaemic cells. After an initial five cycles of low stringency amplification (1 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C), the next 30 cycles of amplification were at higher stringency (1 min at 94 °C, 1 min at 50 °C and 2 min at 72 °C). PCR products were gel-purified, digested with *Bsp*HI and *Bam*HI, and subcloned into a modified pBSK vector (Promega) with an added *Nco*I restriction site. The 5' and 3' DNA sequences were determined using the dideoxy-sequencing method³⁰. The PCR fragment corresponding to *cdk2* was radioactively labelled using the random primer method and used to screen a lambda ZAP II Nalm-6 cDNA library. Three overlapping positive clones, 1.3-kilobases (kb), 1.75-kb and 2.2-kb long, were obtained after two million plaques were screened. The 2.2-kb clone is likely to contain the entire open reading frame of the *cdk2* gene, on the basis of the presence of an ATG initiation codon and sequence alignment with the *cdc2* gene.

to *cdc2* in the 16 amino-acid residue stretch that contains the PSTAIRE sequence, a motif conserved among all *cdc2* genes isolated so far. It also has the conserved phosphorylation sites that are known to be important for regulating *p34^{cdc2}* kinase activity in a cell-cycle-dependent manner: threonine 14 (ref. 15), tyrosine 15 (ref. 16) and threonine 161 (B. Ducommun *et al.*, manuscript submitted).

To investigate whether *cdk2* could encode the cyclin A-associated *p33*, *cdk2* protein was synthesized *in vitro* by translation in a rabbit reticulocyte lysate and compared with the *in vivo*

labelled *p33*. Both *p33*, coprecipitated with anti-cyclin A antibodies, and *in vitro* translated *cdk2* were recognized by anti-PSTAIRE antibodies, but neither protein was immunoprecipitated by antibodies specific for the C-terminal peptide of *p34^{cdc2}* (Fig. 2a). By contrast, *in vitro* translated *p34^{cdc2}* was recognized by both antibodies. From these experiments, we conclude that *p33* and the *cdk2* gene product share immunological properties which are distinct from those of *p34^{cdc2}*. We next subjected the three polypeptides to partial proteolytic mapping to assess similarity at the amino-acid level (Fig. 2b). Digestion of *in vitro* translated *cdk2* with *S. aureus* V8 protease, which cleaves after acidic residues, gave a pattern similar to that obtained by digestion of cyclin A-associated *p33*, whereas digestion of *in vitro* translated *p34^{cdc2}* gave a clearly distinct pattern. Together these data show that the immunological and physical properties of the protein product of the *cdk2* gene are similar to those of cyclin A-associated *p33*.

As there are now a large and growing number of *cdc2*-related cDNA clones, we sought direct evidence to demonstrate that the *cdk2* cDNA could encode *p33*. Mouse antisera specific for *cdk2* were raised against bacterially expressed glutathione-S-transferase-*cdk2* fusion protein. As expected, these antibodies

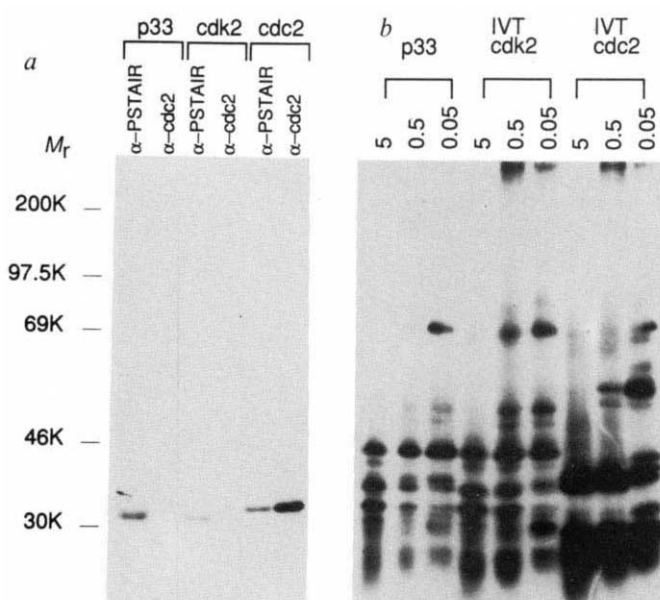
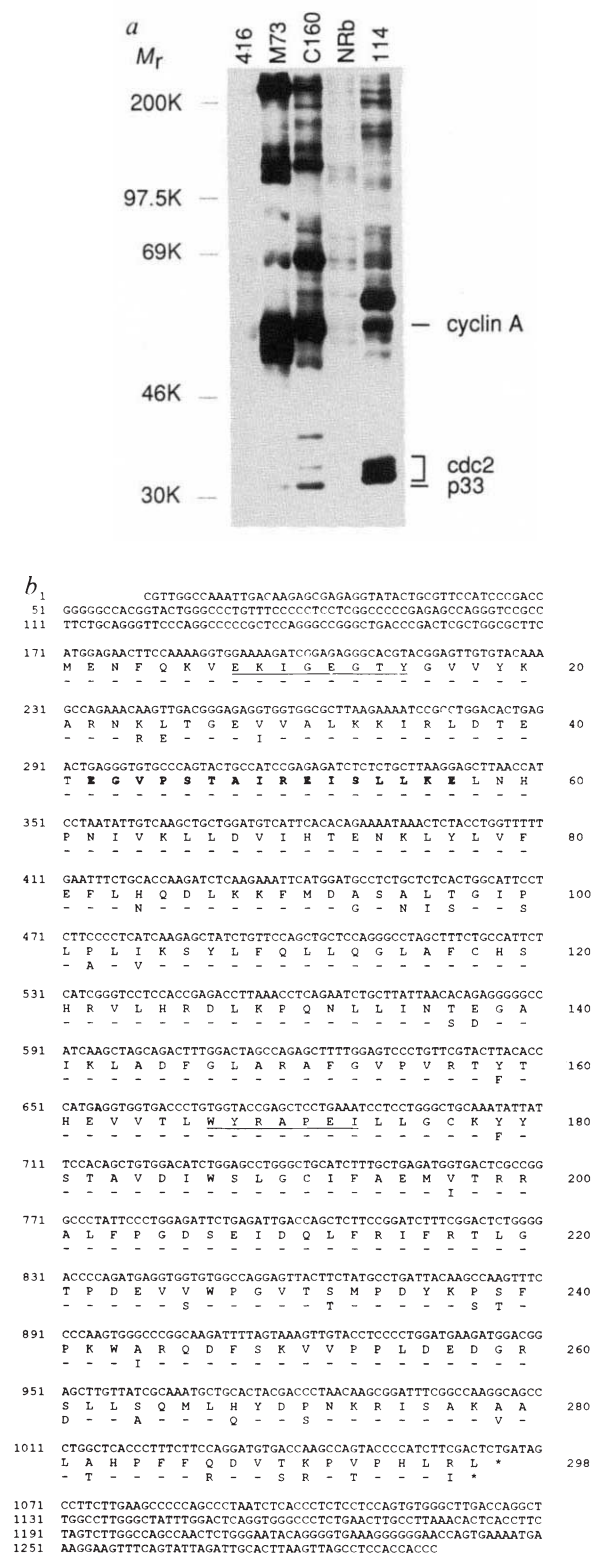


FIG. 2 *a*, Immunological comparison of *p33* and *in vitro* translated *cdk2* protein. The *p33* was obtained by cyclin A immunoprecipitation. Both *cdk2* and *cdc2* were synthesized *in vitro* by translation in a rabbit reticulocyte lysate. All three proteins were purified from SDS-polyacrylamide gels and immunoprecipitated with anti-PSTAIRE antibodies (lanes 1, 3 and 5), or an anti-*cdc2* monoclonal antibody Bcdc2.1 (lanes 2, 4 and 6). *b*, Partial proteolytic mapping of *p33*, *cdk2* and *cdc2*. The *p33* was from lysates of [³⁵S]methionine-labelled ML-1 cells (human myeloid leukaemia) (lanes 1–3), *cdk2* from *in vitro* translations (IVT) of the cDNA clone shown in Fig. 1b (lanes 4–6), and *cdc2* from *in vitro* translations of human *cdc2* cDNA (lanes 7–9) were each treated with three different concentrations (5 μg, 0.5 μg and 0.05 μg) of *S. aureus* V8 protease.

METHODS. Human *cdc2* cDNA was cloned into a pBSK vector for *in vitro* transcription with T7 RNA polymerase (Promega) and pBSK-*cdk2* was transcribed with T3 RNA polymerase (Promega). Both proteins were made by *in vitro* translation in rabbit reticulocyte lysate (Promega). For reprecipitation, [³⁵S]-labelled proteins were separated by SDS-PAGE. The relevant bands were located by autoradiography, excised, and eluted from the gel with 0.1 M NH₄HCO₃ and 0.05% SDS³¹. Eluted proteins were filtered through a Whatman GF/C filter and equal counts were immunoprecipitated with the antibodies shown. *S. aureus* V8 partial proteolysis was done using the method of Cleveland *et al.*³².

were able to immunoprecipitate *cdk2* protein prepared from *in vitro* translation of *cdk2*. But the antibodies failed to recognize authentic p34^{cdc2} either made *in vitro* or *in vivo*, or the gene products of the other *cdc-2*-related clones tested (M.M. and L.-H.T., unpublished data). From lysates of [³⁵S]methionine-labelled cells, these antisera specifically recognized a 32–33K protein band that had many properties reminiscent of p33 (Fig. 3a, lane 3). But this band migrated slightly faster than cyclin A-associated p33 (Fig. 3a, lane 2). As phosphorylation of p34^{cdc2} retards its migration on SDS-polyacrylamide gels, we postulated that the mobility differences between p33 and the anti-*cdk2* immunoprecipitated band might be due to protein modification. Two approaches were used to test this. First, [³⁵S]methionine-labelled immunoprecipitations were treated with potato acid phosphatase, a potent phosphatase for phosphothreonine, phosphoserine and phosphotyrosine residues. After treating the immunoprecipitated proteins with the phosphatase, both the cyclin A-associated p33 (Fig. 3b, lanes 2 and 3) and the 33K protein recognized by *cdk2* antisera (Fig. 3b, lanes 4 and 5)

migrated faster compared with treatment with phosphatase buffer alone. Indeed, dephosphorylated p33 and dephosphorylated *cdk2* protein seemed to comigrate (Fig. 3b, lanes 3 and 5). Second, when immunoprecipitations were done from lysates of [³²P]orthophosphate-labelled cells, the phosphorylated form of the 33K protein recognized by anti-*cdk2* antisera comigrated with the phosphorylated form of cyclin A-associated p33 (Fig. 3c).

Finally, to determine the similarity at a structural level between the 33K protein precipitated directly by the anti-*cdk2* antisera and the p33 associated with cyclin A, these proteins were excised from an SDS-polyacrylamide gel and digested with *S. aureus* V8 protease. Figure 3d shows that when these proteins were both labelled *in vivo*, the digestion patterns were indistinguishable. In addition, a 60K cellular protein, comigrating with cyclin A, was found in anti-*cdk2* immunoprecipitations (Fig. 3a, b). The V8 partial proteolytic map of *cdk2*-associated p60 was identical to that of human cyclin A protein (Fig. 3e). Together, these data suggest that the *cdk2* protein and cyclin A

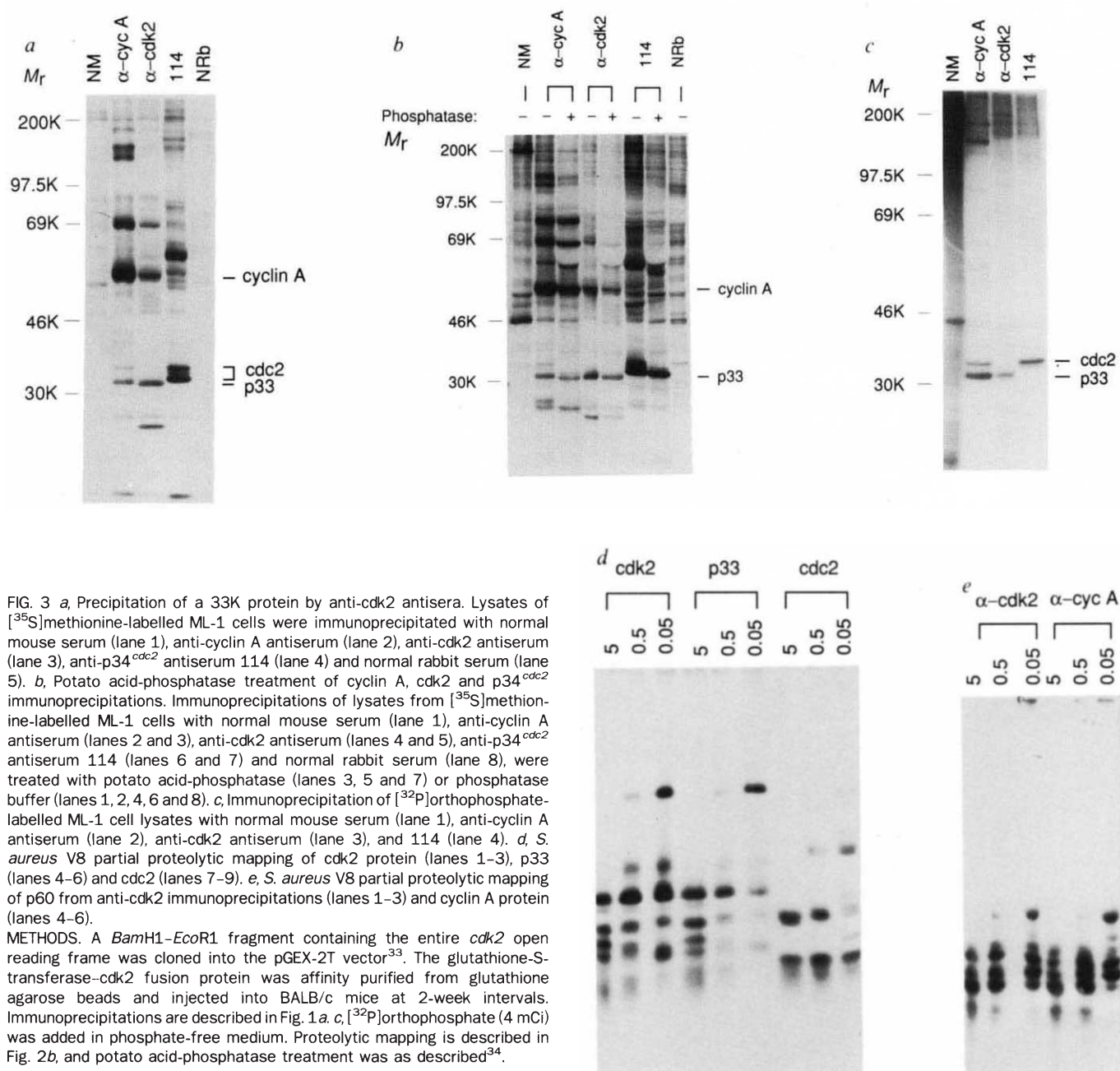
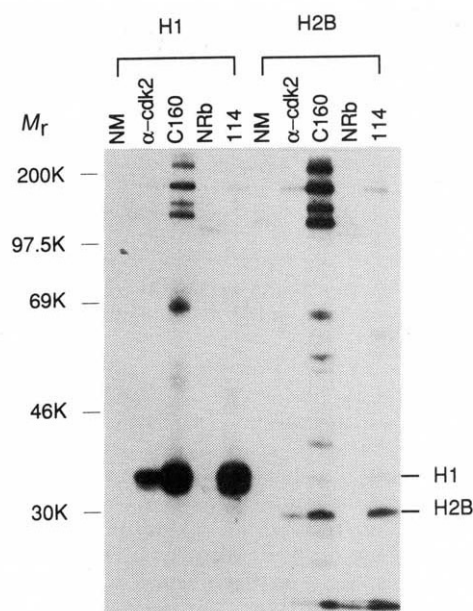


FIG. 3 a, Precipitation of a 33K protein by anti-*cdk2* antisera. Lysates of [³⁵S]methionine-labelled ML-1 cells were immunoprecipitated with normal mouse serum (lane 1), anti-cyclin A antiserum (lane 2), anti-*cdk2* antiserum (lane 3), anti-p34^{cdc2} antiserum 114 (lane 4) and normal rabbit serum (lane 5). b, Potato acid-phosphatase treatment of cyclin A, *cdk2* and p34^{cdc2} immunoprecipitations. Immunoprecipitations of lysates from [³⁵S]methionine-labelled ML-1 cells with normal mouse serum (lane 1), anti-cyclin A antiserum (lanes 2 and 3), anti-*cdk2* antiserum (lanes 4 and 5), anti-p34^{cdc2} antiserum 114 (lanes 6 and 7) and normal rabbit serum (lane 8), were treated with potato acid-phosphatase (lanes 3, 5 and 7) or phosphatase buffer (lanes 1, 2, 4, 6 and 8). c, Immunoprecipitation of [³²P]orthophosphate-labelled ML-1 cell lysates with normal mouse serum (lane 1), anti-cyclin A antiserum (lane 2), anti-*cdk2* antiserum (lane 3), and 114 (lane 4). d, *S. aureus* V8 partial proteolytic mapping of *cdk2* protein (lanes 1–3), p33 (lanes 4–6) and *cdc2* (lanes 7–9). e, *S. aureus* V8 partial proteolytic mapping of p60 from anti-*cdk2* immunoprecipitations (lanes 1–3) and cyclin A protein (lanes 4–6).

METHODS. A *Bam*H1–*Eco*R1 fragment containing the entire *cdk2* open reading frame was cloned into the pGEX-2T vector³³. The glutathione-S-transferase-*cdk2* fusion protein was affinity purified from glutathione agarose beads and injected into BALB/c mice at 2-week intervals. Immunoprecipitations are described in Fig. 1a. c, [³²P]orthophosphate (4 mCi) was added in phosphate-free medium. Proteolytic mapping is described in Fig. 2b, and potato acid-phosphatase treatment was as described³⁴.

FIG. 4 Histone H1 and H2B kinase activity of p33^{cdk2}. ML-1 cell lysates were immunoprecipitated with mouse normal serum (lanes 1 and 6), cdk2 antisera (lanes 2 and 7), C160 (lanes 3 and 8), rabbit normal serum (lanes 4 and 9) and 114 (lanes 5 and 10). The immunoprecipitations were used for kinase reactions in the presence of histone H1 (lanes 1–5) or H2B (lanes 6–10). Phosphorylated H1 and H2B are indicated.

METHODS. Kinase reactions were done as described³⁵. Briefly, after immunoprecipitation, the protein A-Sepharose beads from each immunoprecipitation were incubated with 2.5 µg of the exogenous substrates in the presence of 50 mM HEPES, pH 7.0, 10 mM MgCl₂, 5 mM MnCl₂, 1 mM DTT and 5 µCi [γ -³²P]ATP in a total volume of 50 µl for 20 min at room temperature. The samples were then applied to SDS-polyacrylamide gel and detected by autoradiography.



are associated *in vivo* and that the cyclin A-associated protein, p33, is the product of the *cdk2* gene.

Because p33^{cdk2} contains the characteristic motifs of the cdc2 family of protein kinases and because it associates *in vivo* with cyclin A, which also binds to those of p34^{cdc2}, p33^{cdk2} probably has biochemical activities similar to those of p34^{cdc2}. As p34^{cdc2} phosphorylates histones *in vitro*^{17,18}, we predicted that p33^{cdk2} might also have histone kinase activity *in vitro*. Figure 4 shows that histones H1 and H2B were phosphorylated by anti-cdk2 immunoprecipitations but not by control immunoprecipitations. This experiment indicates that p33^{cdk2}, or the p33^{cdk2}/cyclin A complex, can act as a protein kinase, although the physiological substrates for the p33^{cdk2} kinase are unknown.

Our results demonstrate that cyclin A binds to the *cdk2* gene product at least in human somatic cells, although there has previously been some confusion as to whether the *Xenopus* Eg-1/*cdk2* gene product associates with cyclin A. On the basis of its sequence homology to p34^{cdc2} as well as its association with cyclin A, the p33^{cdk2} kinase is likely to have an important role in cell-cycle progression. Indeed, *cdk2* has been cloned independently and is able to rescue CDC28 mutants of *Saccharomyces cerevisiae*^{19,20}. Although p34^{cdc2} is essential for both the G1 to S and G2 to M transitions in *S. cerevisiae* and

Schizosaccharomyces pombe^{21,22}, only M-phase promoting activity for p34^{cdc2} has been conclusively demonstrated in vertebrate cells^{1–4}. Cyclin A-associated kinase activity appears earlier in the cell cycle than p34^{cdc2}/cyclin B kinase activity (refs 5, 6 and M. Pagano, R. Pepperkok, F. Verde, W. Ansorge and G. Draetta, manuscript submitted), and recent evidence suggests that cyclin A may be required for DNA synthesis (M. Pagano *et al.*, submitted, and C. Br  chot, personal communication). Therefore, p33^{cdk2}/cyclin A complexes may participate in some aspect of S-phase regulation.

It is interesting that the adenovirus E1A proteins selectively target p33^{cdk2} or p33^{cdk2}/cyclin A complexes rather than p34^{cdc2} or p34^{cdc2}/cyclin A complexes. E1A induces cellular DNA synthesis and cell-cycle progression of quiescent cells^{23–25}, and it is intriguing to speculate that the p33^{cdk2}/cyclin A complex might be important for one of the functions of E1A. In other systems, notably the association of E1A with the products of tumour suppressor genes, these viral oncoproteins have led investigators to the identification of important regulatory molecules. Targeting by E1A of proteins, such as p33^{cdk2} and cyclin A, that could regulate aspects of G1 to S transition or DNA synthesis, may provide a clue as to how DNA tumour viral oncoproteins alter normal cell cycle control. □

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Editing of a chloroplast mRNA by creation of an initiation codon

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PRIMARY mRNA transcripts in several systems are edited by single base substitutions, small deletions or insertions to yield functional messenger RNA species^{1,2}. Mitochondrial mRNAs in particular, including those from plants³⁻⁵, seem to be the subject of extensive editing, unlike mRNAs encoded by chloroplast DNA, for which the prediction of amino-acid sequence from the corresponding gene sequence is generally unambiguous⁶⁻⁸. Occasionally, however, an ACG codon appears at the 5' terminus of chloroplast genes, where the initiation codon ATG would be expected. Here we present evidence for a C → U editing that is responsible for the conversion of the ACG codon to an AUG initiation codon in the mRNA transcript from the *rpl2* gene of the maize plastome, showing that mRNA editing can also occur in chloroplasts.

The *rpoA* operon contains the *rpl2* gene in its proximal region and is found in the plastomes of higher plants such as tobacco⁶, liverwort⁷, rice⁸ and maize (unpublished results). As depicted in Fig. 1 for the maize plastome, this operon extends from the

end of the inverted repeat region IR_B into the large single-copy region with the two proximal genes *rpl23* and *rpl2* also being present at the end of the inverted repeat region IR_A. Most genes of the *rpoA* operon, like the majority of chloroplast DNA-encoded genes, have the canonical ATG start codon (GTG in rare cases), but the *rpl2* genes from rice⁸ and maize⁹ chloroplasts have an ACG codon at the position corresponding to an ATG codon in tobacco and liverwort. As no in-frame ATG or GTG codons are found upstream or downstream of this position (see Fig. 1c), we considered here whether this ACG codon is maintained at the mRNA level, or whether it is converted to a functional AUG initiator codon by C → U editing.

We chose the polymerase chain reaction (PCR) primer pair P₁P₃ for amplification of *rpl2* sequences from either chloroplast DNA or a complementary DNA derived from chloroplast RNA. Figure 2 shows that amplification of chloroplast DNA with this primer pair leads to a single product whose size is consistent with the expected 1,170-base pair (bp) fragment containing the intron of the *rpl2* gene (lane l). Amplification of the cDNA, however, leads to a much shorter product of 506 bp, reflecting the loss of the 664-bp intron sequence of spliced *rpl2* mRNA (lane n). A control amplification with the primer pair P₁P₂ from a region upstream of the intron leads to an identical 173-bp product with both DNA and cDNA from chloroplasts (lanes k and m). The size of the amplification products in the case of the primer pair P₁P₃ is indicative of the origin of the products from either the gene or the mRNA.

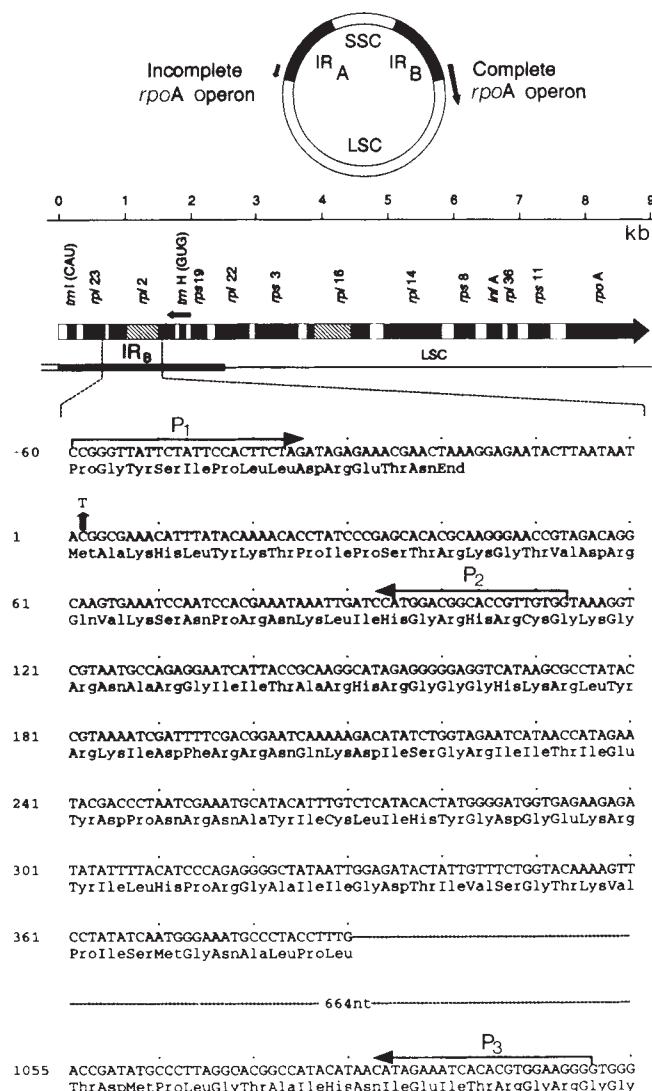
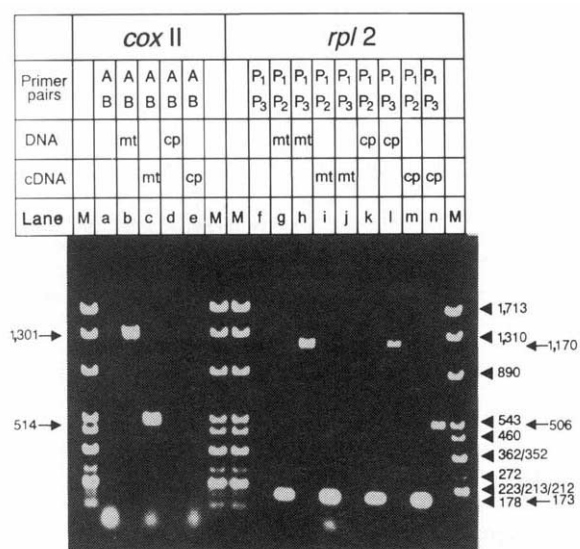


FIG. 1 Position, copy number and structure of the *rpl2* gene of the maize plastome. In *a*, the position is indicated of the *rpoA* operon in relation to the inverted repeat regions IR_A and IR_B and to the small and large single-copy regions (SSC and LSC), respectively, of the maize plastome. The proximal part of the *rpoA* operon which includes the *rpl2* gene is also present on the inverted repeat IR_A. *b*, Structure of the complete *rpoA* operon and the position of the *rpl2* gene on this operon are depicted schematically. Introns of the *rpl2* and *rpl16* genes are marked by striations. The transcription start point of the operon has not been determined and the *trnH* gene is of opposite polarity. *c*, Part of the nucleotide sequence of the *rpl2* gene⁹ including the first exon, the two intron/exon borders and part of the second exon are shown. The positions and orientations of the primers P₁ (5' primer), P₂ and P₃ (both 3' primers complementary to the respective mRNA sequences) used for PCRs are indicated by horizontal arrows. The ACG codon that is edited to the AUG codon is marked by the C-to-T transition.



Sequencing of the two amplification products (Fig. 3) showed that the chloroplast DNA-derived sequence (from the 1,170-bp product in lane l of Fig. 2) agrees with the published sequence⁹, including the ACG codon (Fig. 3, lower panel), but the cDNA has clearly suffered a G-to-A transition (Fig. 3, upper panel) which reflects a conversion of the ACG to the AUG initiator codon in the mRNA. We have confirmed this observation with several amplification products (see Fig. 4, for instance), including the shorter amplification products obtained with the primer pair P₁P₂ (data not shown).

In view of the sensitivity of the polymerase chain reaction and the known transfer of chloroplast genes to the plant mitochondrial genomes¹⁰⁻¹², it was essential to exclude an artefactual result caused by contamination of the chloroplast DNA and RNA preparations with mitochondrial DNA (mtDNA) and RNA, respectively, for which C-to-U editing is well known²⁻⁵. Hence we amplified *coxII* sequences which are specific for the maize mitochondrial genome¹³ from maize mtDNA and cDNA, respectively. As this gene also contains an intron, and as the primer pair used for amplification lies on either side of this intron, the product of mtDNA amplification is large, being of the size expected of 1,301 bp (Fig. 2, lane b); a 514-bp product lacking the intron sequence of 787 bp is formed from the *coxII* mRNA-derived cDNA (Fig. 2, lane c). The

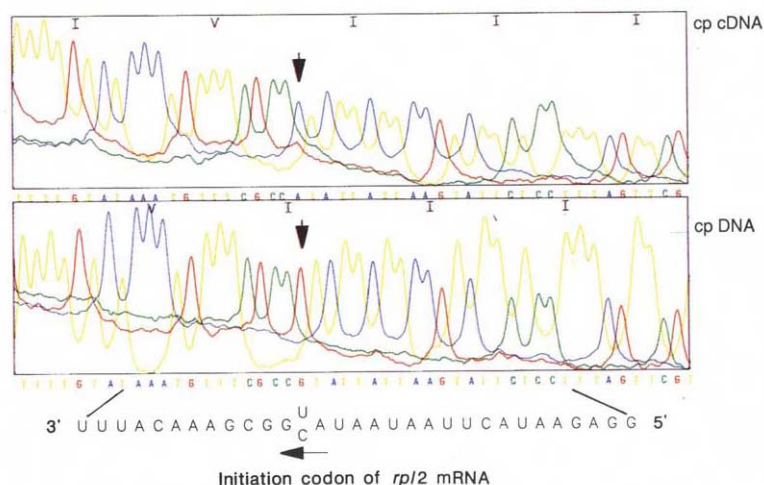
FIG. 2 Amplification products obtained by PCR with the primer pair AB specific for the mitochondrial *coxII* gene and with primer combinations P₁P₂ or P₁P₃ specific for the *rpl2* gene. Amplifications were carried out either with DNA from maize chloroplasts (cp) or maize mitochondria (mt) or with cDNAs obtained by reverse transcription of RNAs from the same organelles. The positions and expected sizes in bp of amplification products are indicated by horizontal arrows; the positions of size markers are given by triangles. METHODS. Sequences and positions of the *rpl2*-specific oligonucleotide primers P₁, P₂ and P₃ are indicated in Fig. 1c. The *coxII*-specific primer A (5'-GCTGCGGAACCATGGCAATTAG3') and B (5'-GAGGTACATCAGCGGGTGTTC3') correspond to positions 94 to 115 and 1,380 to 1,401 of the published *coxII* gene from maize mitochondria^{13,14}. DNA and RNA from maize chloroplasts and from maize mitochondria were isolated as described¹⁵. The two RNA preparations when separated by electrophoresis on a 1.2% formaldehyde-agarose gel gave patterns typical of chloroplast and plant mitochondrial RNAs (data not shown). Chloroplast and mitochondrial RNAs were reverse transcribed with a random primer mixture¹⁷. DNAs and cDNAs were amplified according to a standard protocol with 30 cycles of 92 °C (for 1 min 20s), 55 °C (for 1 min 20s) and 72 °C (for 1 min 20s). Reactions contained magnesium chloride at a final concentration of 1.5 mM. Separation of the products was achieved by electrophoresis on 2% agarose gels sized by comparison with markers (lane M). Control reactions using buffer instead of DNA were run in lanes a and f.

sequences of both products (data not shown) agree with their published sequences¹³, including several edited positions in the cDNA¹⁴. The *coxII*-specific primers were tested in chloroplast DNA and cDNA amplification and gave no products (Fig. 2, lanes d and e), confirming that the chloroplast DNA and RNA used for amplification of the *rpl2* sequences were not contaminated with mtDNA or RNA, respectively. When, on the other hand, the *rpl2*-specific primer combinations were used for amplification of mtDNA or cDNA (Fig. 2, lanes g-j), the same products are formed as with chloroplast DNA and cDNA, with the exception of lane j, which contains only a very faint band, if any, of a 506-bp product (this is probably due to fast degradation of the nonfunctional plastidic *rpl2* mRNA in mitochondria; the terminal fragment responsible for the 173-bp product should have a longer half-life). This formation of *rpl2*-specific amplification products is consistent with the known transfer of a 12-kb fragment¹⁰ and its flanking 5.3- and 4.6-kb fragments¹¹ from the chloroplast DNA to the mtDNA of maize. The two smaller fragments, which include the border sequences between the two inverted repeat regions and the large single-copy region (Fig. 1a), carry the *rpl2* sequences (ref. 15, and our unpublished results).

Because of their sequence divergence and fragmentation, the chloroplast DNA sequences transferred to the mitochondrial

FIG. 3 Comparison of the *rpl2* nucleotide sequences obtained from amplification products of maize chloroplast DNA (lower panel) and cDNA (upper panel).

METHODS. The cDNA sequence was obtained from a 506-bp amplification product (see Fig. 2, lane n) as template after asymmetric amplification in the presence of the primers P₁/P₃ with primer P₃ in limiting concentration. The chloroplast DNA-derived sequence was obtained from a 1,170 bp amplification product (Fig. 2, lane l) as template, also after asymmetric amplification with the same primer combination. The single-stranded products were further purified by chromatography on Qiaquick columns according to the manufacturer's protocol (Qiagen, Düsseldorf). Sequencing was performed by the chain termination method but using a fluorescent primer P₂ for labelling of the products. Products of the sequencing reaction were separated by denaturing PAGE and analysed during electrophoresis by an automated laser fluorescence detection system (EMBL)¹⁸. Owing to the polarity of the sequencing primer P₂, the sequences are complementary to *rpl2* mRNA. The *rpl2* sequence of mRNA polarity with the initiation codon in its chloroplast DNA- and cDNA-derived sequence is depicted at the bottom. The positions where the derived sequences differ by an A-to-G conversion are arrowed.



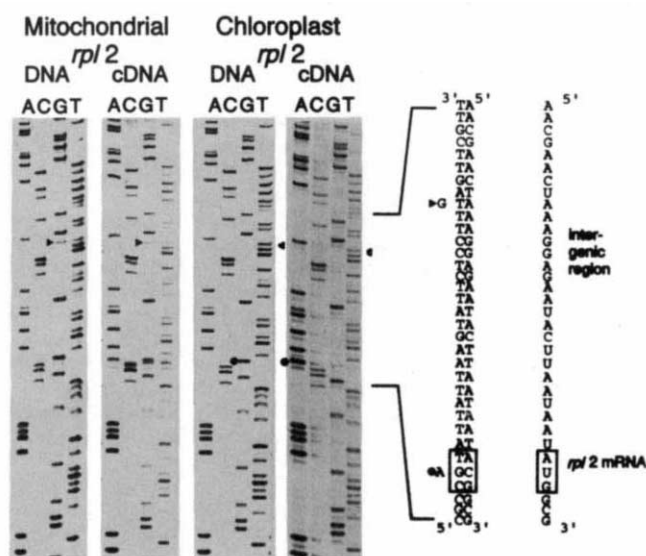


FIG. 4 Sequence analysis of *rpl2* amplification products obtained from mitochondrial and chloroplast DNA and cDNA, respectively. The 173-bp amplification products derived from mitochondrial DNA or cDNA (Fig. 2, lanes g and i) and the 1,170- and 506-bp amplification products derived from chloroplast DNA or cDNA (Fig. 2, lanes l and n) were isolated and sequenced directly by a modified chain termination method¹⁹ using primer P₂ as sequencing primer. Because of the polarity of this primer the sequences of the four autoradiograms are complementary to the *rpl2* mRNA. The editing positions where cDNA- and chloroplast DNA-derived sequences differ are marked by dots. The polymorphic positions where the mitochondrial sequences differ from the chloroplast *rpl2* sequence are marked by triangles.

genome probably do not encode functional genes^{2,11} (with the exception of several transfer RNA genes²). It is evident from the sequence analysis shown in Fig. 4 that the mitochondrial *rpl23/rpl2* intergenic region has a divergent and at the same time polymorphic sequence with respect to the corresponding -20 position of the chloroplast sequence, and this divergence can be used as a marker for discriminating between chloroplast- and mitochondrial-derived *rpl2* sequences. The absence of this polymorphic site in the chloroplast-derived sequence ladders of Fig. 4 (also in the sequence profile of Fig. 3) further excludes a mitochondrial origin. The observed editing must therefore originate in the chloroplast and is not caused by amplification of contaminating mitochondrial *rpl2* sequences. (A partial editing of the same position is also evident in the mitochondrial cDNA in Fig. 4, but we did not consider it relevant here to establish whether this reflects a genuine mitochondrial editing or a contamination of mitochondrial RNA with chloroplast RNA.)

The single mRNA editing position that we have discovered for the chloroplast genetic system is unlikely to be unique. A similar C-to-U editing has been identified in the *psbL* gene of the tobacco plastome (J. Kudla, G.L.I., M. Metzlaß, R. Hagemann and H.K., manuscript submitted). This observation indicates that editing could be a more general process in chloroplast gene expression and that amino-acid sequences as well as open reading frames from chloroplast DNA sequences cannot be deduced from the straightforward rules of the genetic code. □

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Normal development and function of CD8⁺ cells but markedly decreased helper cell activity in mice lacking CD4

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T CELLS express T-cell antigen receptors (TCR) for the recognition of antigen in conjunction with the products of the major histocompatibility complex^{1,2}. They also express two key surface coreceptors, CD4 and CD8, which are involved in the interaction with their ligands^{3,4}. As CD4 is expressed on the early haemopoietic progenitor⁵ as well as the early thymic precursor cells⁶, a role for CD4 in haemopoiesis and T-cell development is implicated. Thymocytes undergo a series of differentiation⁷ and selection steps^{8,9} to become mature CD4⁺8⁻ or CD4⁻8⁺ (single positive) T cells^{10,11}. Studies of the role of CD4⁺ T cells *in vivo* have been based on adoptive transfer of selected or depleted lymphocytes, or *in vivo* treatment of thymectomized mice with monoclonal antibodies causing depletion of CD4⁺ T cells¹²⁻¹⁴. In order to study the role of the CD4 molecule in the development and function of lymphocytes, we have disrupted the CD4 gene in embryonic stem cells^{15,16} by homologous recombination^{17,18}. Germ-line transmission^{19,20} of the mutation produces mutant mouse strains that do not express CD4 on the cell surface. In these mice, the development of CD8⁺ T cells and myeloid components is unaltered, indicating that expression of CD4 on progenitor cells and CD4⁺ CD8⁺ (double positive) thymocytes is not obligatory. Here we report that these mice have markedly decreased helper cell activity for antibody responses, although cytotoxic T-cell activity against viruses is in the normal range. This differential requirement for CD4⁺ helper T cells is important to our understanding of immune disorders, including AIDS, in which CD4⁺ cells are reduced or absent.

The CD4 molecule is a T-cell-specific surface glycoprotein of relative molecular mass 55,000 (*M_r*, 55K)¹¹. For homologous recombination, a replacement-type vector (pML3T4Neo) was made (Fig. 1a). This DNA construct was introduced into D3 embryonic stem (ES) cells by electroporation. Eight independently targeted lines of ES cells were generated (data not shown). The average frequency of homologous recombination was about 1 in 2 × 10⁷ electroporated cells or 1 in 300 G418-resistant clones.

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Southern blot analysis confirmed the homologous recombination event (Fig. 1b). Germ-line transmission of the mutation occurred in mice representing four independent clones named 32.3, 39.2, 58.8 and 59.3 (data not shown). Heterozygous mice from three of these lines were interbred to obtain mice homozygous for the disrupted CD4 gene (Fig. 1c; data from 39.2- and 59.3-derived mutant alleles not shown).

Mice, homozygous for the 32.3- and 39.2-derived mutant CD4 alleles were apparently healthy, fertile and indistinguishable from heterozygous or wild-type littermates on gross physical inspection, with no alteration in the size of the lymphoid organs. The numbers of T and B cells, as identified by antibodies specific for Thy-1.2 and IgM, respectively, in lymph nodes of these mice were found to be normal by flow cytometric analysis (data not shown). Myeloid differentiation, as measured by colony-forming units in culture of bone-marrow cells was also unaltered (data not shown).

As expected, surface expression of CD4 was not detected on thymocytes or lymph node cells from homozygous mutant mice (Fig. 2a, b). CD8⁺ cells are present in normal numbers in the thymus but have expanded in the periphery to apparently occupy the compartment that would otherwise have been occupied by CD4⁺8⁺ cells. Also present in the lymph nodes are some cells

that are TCR $\alpha\beta$ ⁺CD4⁻8⁻. CD3 and TCR $\alpha\beta$ expression is normal on thymocytes of the homozygous mutant mice, with no change in the proportion of cells with high and low levels of TCR $\alpha\beta$ expression, suggesting normal maturation of the CD8⁺ T cells.

The production of interleukin-2 (IL-2) in response to allo-antigens was greatly reduced in homozygous mice but some interleukin 2 (IL-2) was still produced (Fig. 3a). To determine whether IL-2 was being secreted in response to class I or class II major histocompatibility complex (MHC) differences, spleen cells from these mice were stimulated with allogeneic cells which differed in class I MHC (H-2^{bm1}), class II MHC (H-2^{bm12}) or both (H-2ⁱ). The amount of IL-2 secreted by homozygous mice in the responses against class II differences was much lower than that secreted by heterozygous mice. Higher amounts of IL-2 were found in homozygous mice in responses against class I MHC difference.

The CD4-deficient mice mounted a poor response against sheep erythrocytes (SRBC). Sera from homozygous mice showed markedly reduced amounts of anti-SRBC antibodies (titre of 1 out of 80) compared with those found in heterozygous or wild-type mice (titre of 1 out of 1,280). *In vitro* plaque-forming assays demonstrated that the spleens of homozygous mice had markedly reduced frequencies of plasma cells producing anti-

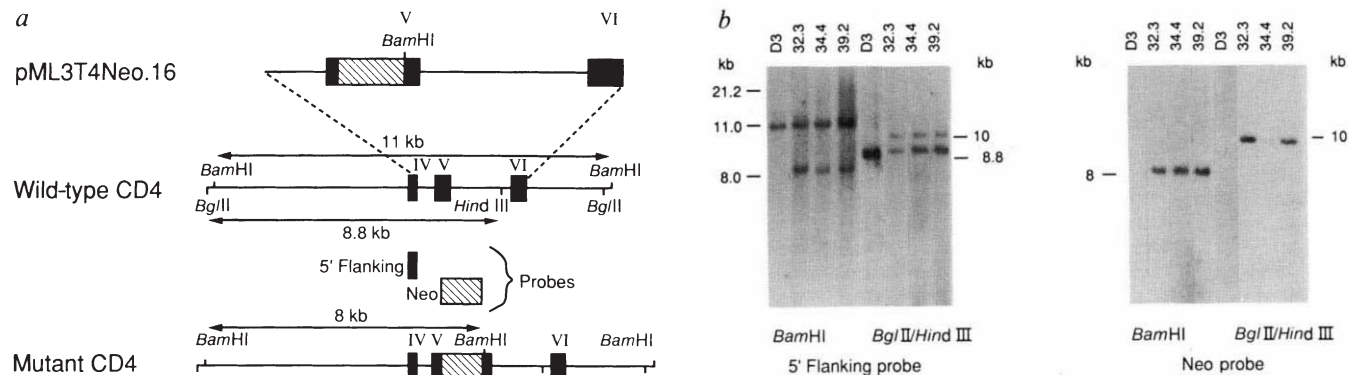
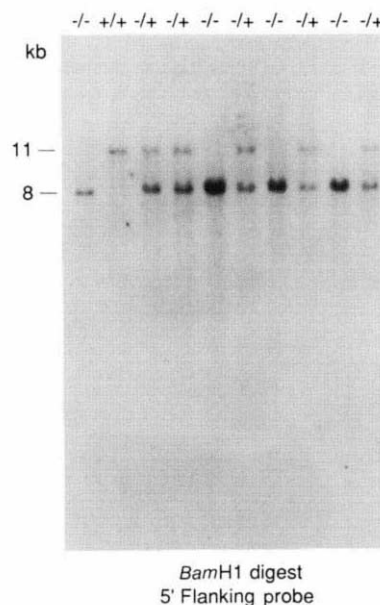


FIG. 1 Gene targeting. *a*, Schematic structure of the targeting vector (pML3T4Neo), the CD4 locus in parental D3 cells (wild-type CD4), and the predicted structure of the targeted CD4 locus (mutant CD4). The neomycin phosphotransferase gene (*neo*) cassette contains the 1.2-kilobase (kb) *Xho*I–*Sal*I fragment of plasmid pMCIPolA (ref. 18) and was inserted in the *Sal*I site created at a previous *Kpn*I site in exon V of the 2.8-kb genomic fragment of CD4. D3 embryonic stem cells from 129/sv mice were maintained in the undifferentiated state either by growth on feeder layer of mitomycin C-treated primary embryonic fibroblasts or in culture medium supplemented with leukaemia inhibitory factor³². Electroporation of ES cells and screening for homologous recombination were carried out as described³³. A primer specific for the *neo* cassette within the herpes simplex virus *tk* promoter, (5'-ATTGCGCAATGACAAGACGCTGG-3'), and another primer specific for the CD4 gene in exon IV and 5' of the targeting vector (5'-GAGGTTCGCCTTCGACGTTTGTAT-3') were used in the polymerase chain reaction (PCR). CD4 cDNA was used as the hybridization probe for the PCR screening. *b*, Southern blot analyses of the structure of the CD4 locus in the DNA of parental D3 cells and three targeted clones 32.3, 34.4 and 39.2. Hybridization probes as in *a*. DNA (10 µg) was digested with the indicated enzymes, run on a 0.8% agarose gel, and blot-hybridized by standard procedures. *c*, Genotypic identification of offspring resulting from interbreeding of 32.3-derived, heterozygous mice. Chimaeric mice were generated by injecting the targeted ES clone into 3.5-day preimplantation embryos derived from superovulated C57BL/6J females and transferring them into 2.5-day pseudopregnant CD1 females. Germ-line transmission of the targeted CD4 alleles was assessed by breeding the chimaeric males with (C57BL/6 × 6DBA/2) F1 females or C57BL/6 females and checking for the ES cell-derived agouti coat colour. Southern blot analysis was carried out on DNA isolated from the tails. 5' flanking probe was as in *a*. The genotypes (+/+, wild-type; +/-, heterozygous; --, homozygous) are indicated.

c Heterozygous x heterozygous mating mouse tail DNA



SRBC antibody (Fig. 3b). A normal response to lipopolysaccharide excluded any intrinsic defect in the B cells of the homozygous mice (data not shown).

Murine cytotoxic T cells are considered to be exclusively $\text{TCR}_{\alpha\beta}^+ \text{CD4}^- 8^+$. To test whether this population could mount a cytotoxic response against viruses, these mice (H-2^b) were infected intravenously with either lymphocytic choriomeningitis virus or vaccinia virus. Homozygous mutant mice generated cytolytic T-cell activity against both viruses that was either comparable with or within a two- to fivefold range of that generated by wild-type and heterozygous mice (Fig. 4; data for wild-type mice not shown).

Our data show that the absence of CD4 expression in the mutant mice results in apparently normal haemopoiesis, indicating that the low expression of CD4 on the surface of an early haemopoietic progenitor cell⁵ and on the surface of the thymocyte precursors⁶ is not required for either haemopoiesis or lymphopoiesis. The results demonstrate that the expression of CD4 on the $\text{CD4}^+ 8^+$ thymocyte is not obligatory for the selection of $\text{CD4}^- 8^+$ cells. Conversely, we have recently shown that sur-

face expression of CD8 on the immature thymocytes is not obligatory for the selection of $\text{CD4}^+ 8^-$ cells²¹.

This study also shows that the absence of CD4 expression in the mutant mice greatly reduced the development of class II MHC-restricted helper T cells. The presence of CD4 on the surface of the thymocytes favours the development and positive selection of functional class II MHC-restricted T cells. Surprisingly, there is a population of $\text{TCR}_{\alpha\beta}^+ \text{CD4}^- 8^-$ cells in the lymph nodes of these mice and the functional significance of these cells is being investigated.

CD4^+ cells regulate antibody production^{22,23}, and the inability of the CD4-deficient mice to mount an efficient anti-SRBC response confirms this. The role of T help in the induction of a cytotoxic T-cell response depends on the virus studied. Some *in vivo* experiments suggest a mandatory role for T help²³⁻²⁷, whereas others show that T help is not necessary for class I MHC-restricted T-cell responses *in vivo*²⁷⁻³⁰. These experiments demonstrate the ability of $\text{CD4}^- 8^+$ T cells to mount an effective anti-viral cytotoxic response in the absence of CD4^+ helper T cells. The CD8^+ cells that develop in the absence of CD4 can

FIG. 2 Flow cytometric analyses of *a*, thymocytes and *b*, lymph node cells from 6-week-old wild-type (+/+) mice and mice heterozygous (+/-) and homozygous (-/-) for the 32.3-derived disrupted CD4 gene. Single-cell suspensions from thymus and lymph nodes of 6-week-old mice were made and $2-5 \times 10^5$ cells were stained with monoclonal antibodies for 45 min at 4 °C in 100 μl PBS containing 1% BSA and 0.1% sodium azide. Cells were then washed and analysed for double-colour flow cytometric analysis on a FACScan (Becton Dickinson). Monoclonal antibodies used were anti-Lyt-2 (53-6.7, Becton Dickinson), anti-L3T4 (GK1.5, Becton Dickinson), anti-CD3 (145-2C11, PharMingen), anti- $\alpha\beta$ TCR (H57-597, PharMingen).

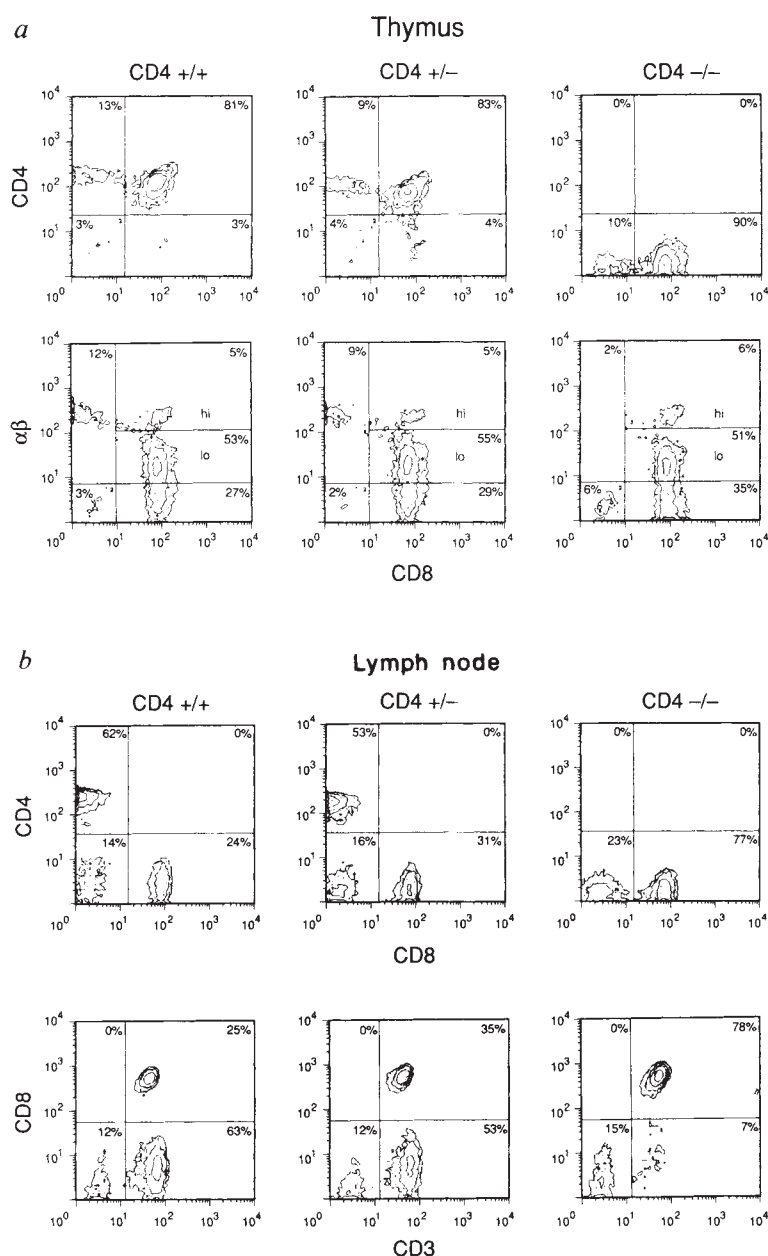


FIG. 3 Assays for helper function. *a*, MHC class restriction for IL-2 release in mixed lymphocyte reaction. Primary bulk mixed lymphocyte reaction cultures were set up. Responder cells were spleen cells from adult (6-8-week-old) mice heterozygous (+/-, solid bars) and homozygous (-/-, hatched bars) for the 32.3 derived mutant allele. These mice were of haplotype H-2^b. The stimulator cells were from mouse strains C57BL/6J (H-2^b), B10.RIII(71NS) (H-2^k), C57BL/6 bm1 (H-2^{b^m1}) or C57BL/6 bm12 (H-2^{b^m12}). Responder cells (2×10^7) were cultured with 2×10^7 irradiated (2000cGy) stimulator cells in 20 ml culture medium. On day three, IL-2 activity in 100 μ l supernatant was determined using the CTLL-assay³⁴. *b*, SRBC-specific plasma cell enumeration. Wild-type (+/+), heterozygous (+/-) and homozygous (-/-) mice were injected intraperitoneally with 3×10^6 sheep erythrocytes (three mice per group) 6 days later, single-cell suspensions from the spleen were made and the number of plasma cells producing antibody (mainly IgM) specific for SRBC were counted using the Jerne Plaque assay³⁵.

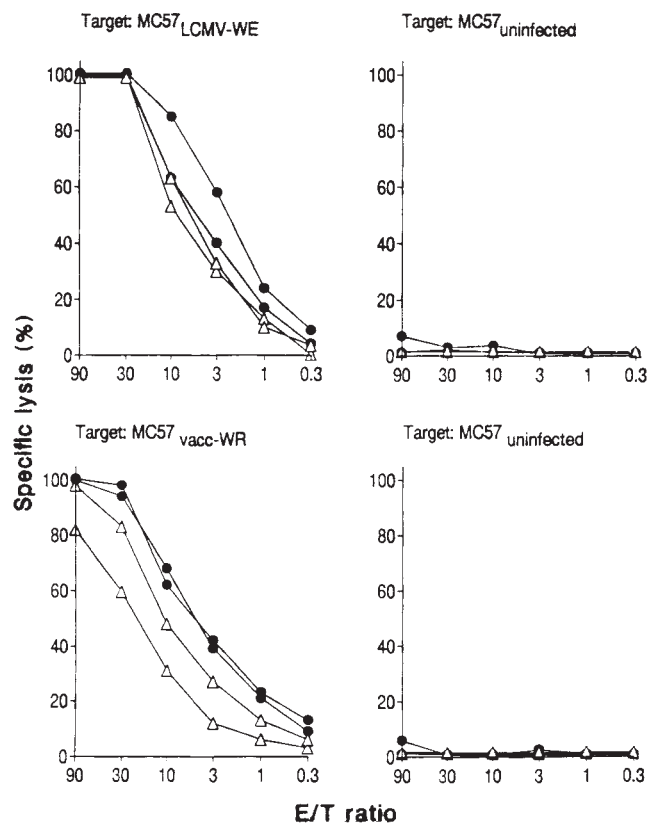
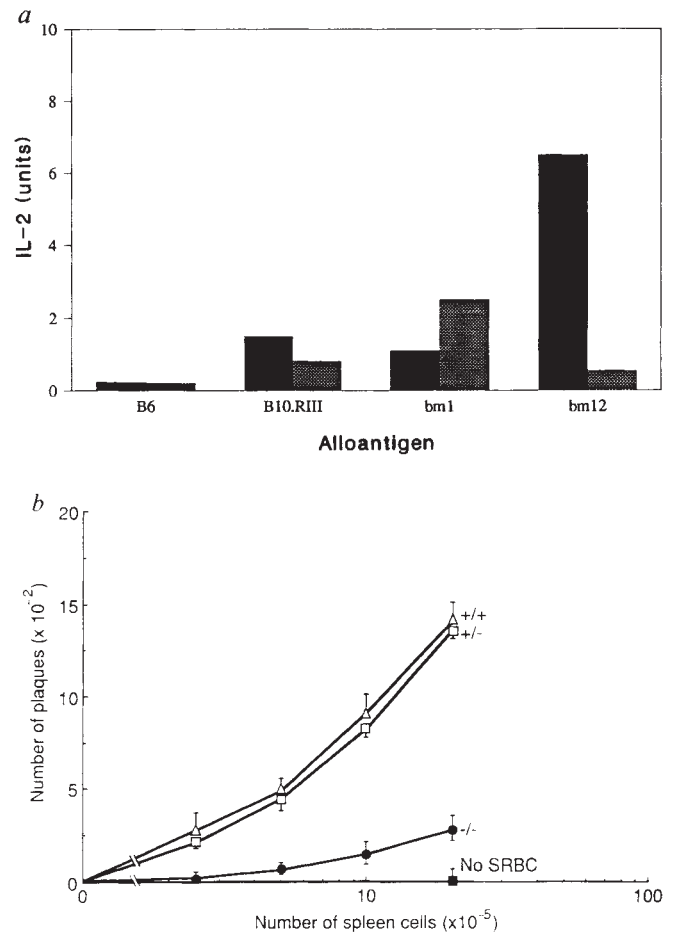


FIG. 4 Cytotoxic activities of T cells from spleens of mice heterozygous (+/-) and homozygous (-/-) for the 32.3 derived mutant CD4 allele. The anti-viral cytotoxic activity of T cells was tested in a standard ⁵¹Cr release assay using MC57G(H-2^b) and natural killer cell-sensitive YAC as target cells. Virus-infected targets were prepared by incubating the cells with the virus and the Na⁵¹CrO₄ for 1 h at 37 °C. The target cells were washed three times with medium containing fetal calf serum. Two adult mice were infected intravenously with either lymphocytic choriomeningitis virus (LCMV) or vaccinia virus. Cytotoxicity assays were carried out 6 days after injection of 2×10^6 plaque forming units of vaccinia virus and 8 days after injection of 2×10^2 plaque forming units of the LCMV strains. Single-cell suspensions of spleen cells were assayed in duplicate. Effector and target cells (1×10^4) were added in a total volume of 0.20 ml to round-bottomed 96-well plates to yield effector to target ratios of 90:1, 30:1, 10:1, 3:1, 1:1 and 0.3:1. After mixing, the plates were centrifuged at 400g for 5 min and incubated at 37 °C in air containing 5% CO₂. The percentage specific ⁵¹Cr was calculated as (experimental release-spontaneous release)/(total release-spontaneous release) $\times 100$. The genotypes (+/-, heterozygous; -/-, homozygous) are indicated. +/-, $\bullet$; -/-, Δ .

secrete IL-2 in response to class I MHC difference and may be a potential source of 'help' (ref. 31). Alternatively, double negative TCR $_{\gamma\delta}^+$ or TCR $_{\alpha\beta}^+$ cells may be responsible for providing it. The finding of a relatively normal cytotoxic T-lymphocyte response against viruses in the absence of CD4 $^+$ helper cells clearly has important implications for our understanding of the role of CD4 $^+$ cells in *in vivo* immune reactions.

This mutant mouse strain has not only provided us with a model to study ontogeny and functions of the immune system, but also for the immunological consequences of diseases such as AIDS, where the CD4 $^+$ cells are reduced or absent. These mice should also provide valuable information about the role of the CD4 $^+$ cells in fighting infections, in tumour and allograft rejection, in the pathogenesis of graft-versus-host disease and in autoimmune diseases, particularly ones in which auto-antibodies are generated. □

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Role of the *Drosophila patched* gene in positional signalling

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AFTER cellularization of the *Drosophila* embryo, positional differences within each primordial segment are maintained and elaborated by processes that require cell interactions. The best-documented examples^{1,2} of such intercellular signalling are the mutual interactions between neighbouring cells expressing the homeodomain protein *engrailed*³ and the secreted glycoprotein encoded by *wingless*⁴, the *Drosophila* homologue of the murine *Wnt-1* gene⁵. Little is known about the molecular basis of these signalling mechanisms but the activities of several other genes, notably *patched* and *hedgehog*, have been implicated in the process^{1,2}. Here we show that the role of *patched* in positional signalling is permissive rather than instructive, its activity being required to suppress *wingless* transcription in cells predisposed to express the latter. According to this view, expression of *wingless* is normally maintained only in those cells receiving an extrinsic signal, encoded by *hedgehog*, that antagonizes the repressive activity of *patched*. We suggest that the *patched* protein may itself be the receptor for this signal, implying that this is an unusual mechanism of ligand-dependent receptor inactivation.

During normal embryogenesis in *Drosophila*, the spatially restricted expression of the *engrailed* (*en*) and *wingless* (*wg*) genes serves to demarcate the anterior and posterior margins respectively of each parasegment^{3–7}. The establishment of these patterns of expression occurs at the blastoderm stage of development in response to cues generated by the pair-rule genes^{8–10}, but their subsequent maintenance depends on mutual cell–cell interactions: in the absence of either gene, the expression of the other ceases shortly after gastrulation^{1,2}, resulting in the disruption of the polarity and integrity of each parasegment¹¹.

The molecular structure and distribution of the *wg* protein^{4,5} suggests that it may itself act as the intercellular signal that maintains *en* transcription in neighbouring cells. That only cells

posterior to *wg* protein-secreting cells respond by transcribing *en* could reflect differences between cells in their capacities to respond to the *wg*-encoded signal. In embryos mutant for the segment-polarity gene *patched* (*ptc*), the expression domain of *wg* broadens anteriorly after gastrulation and transcription of *en* is subsequently induced *de novo* in cells anterior to these broadened domains^{1,2} (Fig. 1).

As the *en* induction depends on *wg* activity², this aberrant cellular behaviour could mean that the activity of *ptc* is instrumental in determining the response of cells to the *wg* signal, the expression of *ptc* protein in some way preventing the signal from being transduced to activate *en*^{12–14}. Alternatively, the ectopic induction of *en* could be a secondary effect of *ptc* mutations, caused by the novel juxtaposition of cells that results from the expansion of the *wg* domain^{1,15}; in this case, the principal function of *ptc* would be to regulate the spatial expression of *wg* by selectively repressing its transcription.

Using polyclonal antisera raised against bacterial fusion proteins, we have analysed the distribution of *ptc* protein during embryogenesis. Like the *ptc* transcript^{13,14,16}, the protein accumulates both in *wg*-expressing as well as non-expressing cells, but is absent from cells expressing *en*. This pattern of expression tends to support the former interpretation of *ptc* function, absence of *ptc* protein from cells being closely correlated with the expression of *en*.

To investigate the significance of this spatial regulation, we tested the consequences of overexpressing *ptc* protein using a *HSptc* gene in which *ptc* expression is directed by the heat-shock protein hsp70 promoter (Fig. 2). Surprisingly, no difference in survival was seen between control and experimental animals following ubiquitous expression of *ptc* protein either during or after gastrulation, and experimental larvae showed no defects other than those observed amongst controls. The expression of *wg* and *en* in *HSptc* embryos 60 min after induction was analysed by *in situ* hybridization; no change in the expression pattern of either gene could be detected (Fig. 2c, d).

To determine whether the *ptc* protein encoded by the construct is functional, we tested its ability to rescue two different *ptc* mutant allelic combinations. As the absence of *ptc* activity also results in ectopic *ptc* expression¹⁴, the patterns of both *wg* and *ptc* were analysed in embryos that were heat-shocked for 30 min between stages 8 and 9 of development (staging according to

ref. 17) and the patterns of *wg* and *ptc* transcription analysed by *in situ* hybridization. In both cases a normal pattern of expression was observed in most *ptc* mutant embryos following the treatment (see Fig. 2 and legend for details).

These experiments indicate that ubiquitous expression of *ptc* protein is capable of rescuing the *ptc* phenotype, and that its sustained expression during and after gastrulation has no effect on the normal cellular interactions that occur at this time. In particular, expression of *ptc* does not appear to compromise the ability of cells to maintain *en* expression in response to the *wg* signal. Instead, it seems that the principal function of *ptc* is to repress *wg* transcription.

Although it might seem paradoxical that the two proteins are co-expressed if *ptc* acts as a repressor of *wg*, we reasoned that *ptc* could be a component of the signalling pathway by which the neighbouring *en* cells maintain *wg* expression; in this case, transduction of the putative signal might stimulate *wg* transcription by antagonizing the repressive activity of *ptc*. Whereas the homeodomain protein encoded by *en* is itself unlikely to act as an intercellular signal maintaining *wg* transcription, a good candidate for such a molecule is the product of the *hedgehog* (*hh*) gene. Expression of *hh* is restricted to *en*-expressing cells (J. Mohler, personal communication) and in embryos lacking *hh* activity, *wg* expression in the epidermis decays during stage 10 (ref. 14), resulting in a phenotype similar to that of *wg* (refs 11, 18; Fig. 3a).

To test our idea that *wg* expression is maintained by antagonizing *ptc* activity, we generated embryos mutant both for *ptc* and for *hh*. In contrast to their *hh* sibs, in which *wg* transcript decays in epidermal cells by stage 10, expression of *wg* in the *ptc hh* double mutants persists in broad domains,

typical of embryos mutant for *ptc* alone. This uncoupling of *wg* expression from *hh* activity is reflected in the suppression of the *hh* phenotype seen in the larval cuticle of the double mutants (Fig. 3; E. Wieschaus, personal communication).

Although *hh* has not yet been fully characterized at the molecular level, an attractive possibility is that it encodes a ligand, the receptor for which is the *ptc* protein. Sequence analysis of *ptc* predicts that it encodes a multipass integral membrane protein^{13,16} and, consistent with this, we find that the *ptc* protein accumulates predominantly around the periphery of each expressing cell (Fig. 1d). According to this interpretation, *ptc* would normally be constitutively active, being specifically inactivated by binding of the putative *hh* ligand (see Fig. 3c). We note, however, that overexpression of the *ptc* protein has no effect on the normal expression of *wg*; this might be because *hh* protein is present in vast excess; alternatively, the binding of a single *hh* protein molecule might be sufficient to inactivate many *ptc* molecules by promoting the formation of inactive multimeric complexes.

Such an antagonistic effect of *hh* on *ptc* function can also account for the way in which the transcription of *ptc* is itself regulated (see Fig. 4). After stage 10, the selective repression of *ptc* transcription in each parasegment limits its expression to two stripes of cells flanking each *en* domain^{13,14,16}. As the activity of *ptc* is required for its own repression¹⁴, *ptc* transcription should rapidly disappear in all cells except those in which its activity is antagonized, that is, in cells adjacent to each *en* domain, exactly as observed. It is notable that whereas the *hh* signal can therefore apparently be received and transduced by cells on either side of the *en* domain, the outcome of this signal depends on the position of the receiving cells. This implies that,

FIG. 1 a, Expression domains of *en* and *wg* during normal embryogenesis. To visualize the expression of both genes simultaneously, we used embryos heterozygous for a chromosome carrying a *lacZ* insertion in the *wg* gene (provided by N. Perrimon). This insertion inactivates *wg* but the mutation is completely recessive. Expression of *en* protein (blue) was revealed using a rat anti-*en* protein antibody (A.M.T., unpublished), whereas the *wg* expression domain (brown) was visualized with a rabbit anti- β -galactosidase antibody. As both the proteins are expressed cell-autonomously, the staining patterns reflect the transcriptional domains of each gene. Note that these are mutually exclusive but adjacent to one another. b, Expression of *en* and *wg* in a *ptc*⁻ embryo. The same *wg lacZ* insertion was recombined onto a chromosome carrying the deficiency *ptc*^{G12} and flies heterozygous for this chromosome were crossed to *ptc*^{G12} heterozygotes to generate *ptc*⁻ embryos. These were fixed and stained exactly as described in a. Note that the domain of *wg* expression is significantly broader than in wild type; anterior and adjacent to these broadened domains, cells ectopically express *en* protein (arrowheads). c, Distribution of *ptc* protein in an early stage-10 embryo visualized using an anti-*patched* antibody raised in mouse. The pattern is indistinguishable from that of the transcript at the same stage. Note the absence of protein from cells that express *en* at the anterior of each parasegment and its presence in *wg*-expressing cells at the posterior of each parasegment. d, Detail of the embryo in c, showing the predominantly peripheral staining, which is consistent with a localization of the *ptc* protein in the plasma membrane. Note also the presence of intense 'dots' of staining in some cells which may represent a local accumulation of protein at the cell surface.

METHODS. Antibodies against the *ptc* protein were raised in mice and rats using fusion proteins produced by recombinant pGEX or pAR expression plasmids containing a 1.4-kilobase *Bam*H1 fragment from the 5' end of the *ptc* complementary DNA. Fusion proteins were purified by PAGE, eluted from gel slices and injected following standard procedures. A more detailed description of the production and characterization of these and other *ptc* antibodies will appear elsewhere (A.M.T., Y.N. and P.W.L., manuscript in preparation). Embryos were collected, aged and fixed in 4% formaldehyde in phosphate-buffered salts for 15 min before removal of their vitelline membranes in heptane/methanol. After overnight incubation with the primary antibody, embryos were washed, incubated with appropriate secondary antibodies (horse radish peroxidase-conjugated anti-rabbit IgG alkaline phosphatase-conjugated anti-rat IgG, horseradish peroxidase-conjugated anti-mouse IgG; all from Jackson) and stained using an Immunoselect kit (BRL) for alkaline phosphatase detection, and diaminobenzidine for horseradish peroxidase detection. In the case of the mouse anti-*ptc* protein antibody, the diaminobenzidine reaction product was enhanced using cobalt and nickel ions⁴. Stained embryos were mounted in glycerol or JB-4 (Polysciences) and photographed using differential interference contrast optics on a Zeiss Axioplan microscope.

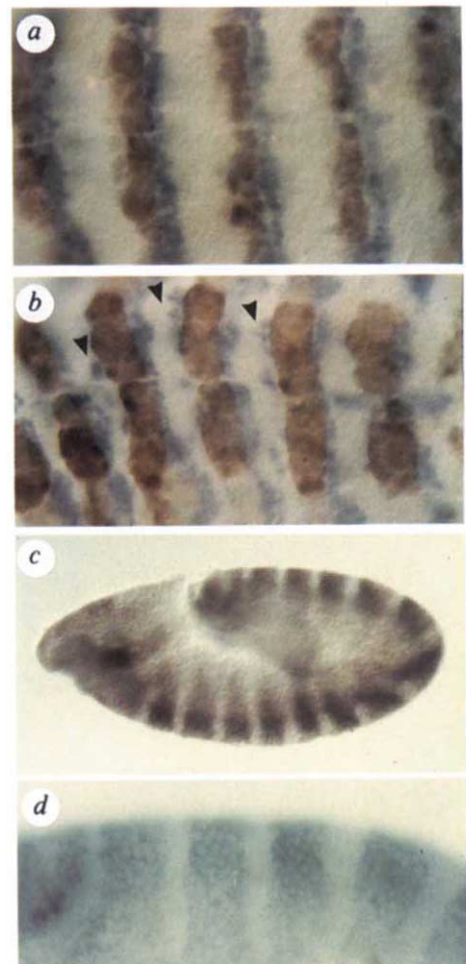
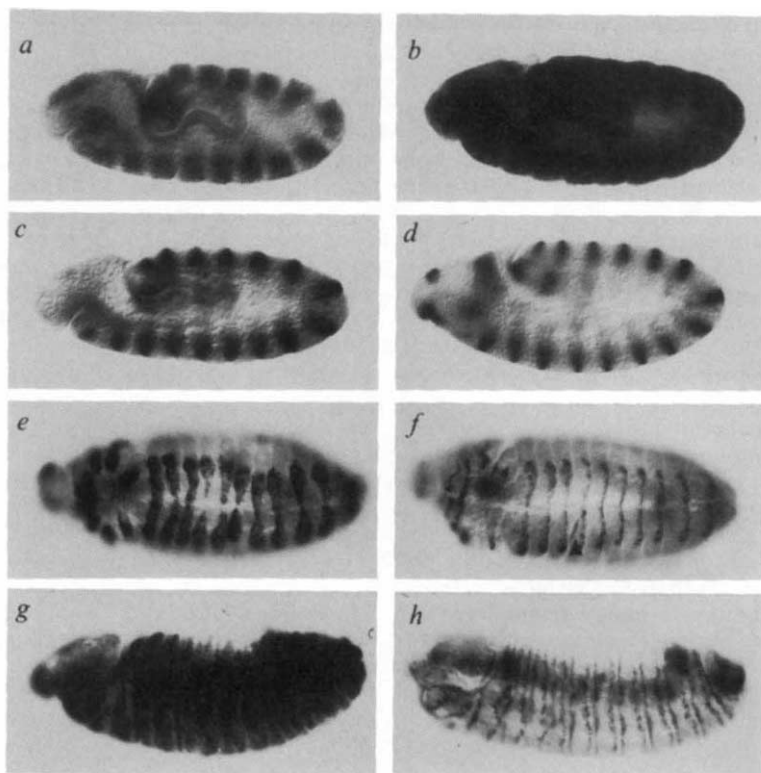


FIG. 2 Distribution of *ptc* protein in *a*, wild type and *b*, *HSptc* stage-10 embryos fixed 30 min after a 30-min heat shock during stage 8/9 of embryogenesis. Note the ubiquitous distribution of protein in the transgenic embryo. *c*, Normal distribution of *en* transcript in *HSptc* embryo fixed 60 min after heat shock. *d*, Normal pattern of *wg* transcript in a *HSptc* embryo fixed 60 min after heat shock. *e-h*, Rescue of *ptc*⁻ phenotype by ubiquitous expression of the *HSptc* construct. *e-f*, Embryos were collected from the cross *HSptc* *F11*/+; *Df(2R)ptc*^{G12}/+X*Df(2R)ptc*^{G12}/+, and were heat-shocked for 30 min between stages 8 and 9, fixed 1 h or 4 h later and hybridized simultaneously with digoxigenin (DIG)-labelled probes for *ptc* and *wg*. One quarter of these embryos are homozygous for the *ptc* deficiency and half of these carry the *HSptc* construct. Hybridizing with *ptc* allowed us to identify unambiguously the *ptc* deficiency homozygotes as they have no endogenous *ptc* expression. The pattern of *wg* expression was analysed in detail in embryos fixed at stage 12/13. Of 86 *ptc*^{G12} embryos, 42 showed the broad stripes of *wg* typical of *ptc* mutants (*e*). Amongst the other 44 embryos, which we presumed were carrying the *HSptc* construct, 30 showed a pattern indistinguishable from wild-type; the remainder, classified as partially rescued, showed normal *wg* expression in most segments, with occasional groups of cells (arrowhead) in one or two segments ectopically expressing *wg* (*f*). *g-h*, Endogenous *ptc* expression in *ptc* mutant embryos with or without ectopic *ptc* expression was analysed using a mutant *ptc* allele associated with an insertion of an enhancer trap vector (gift from K. Jepson-Innes and C. Goodman). Embryos carrying the latter allele, *ptc*^{H84}, express the *lacZ* gene in a pattern essentially identical to the normal *ptc* transcription pattern; this allowed us specifically to monitor endogenous *ptc* expression following expression of the *HSptc* construct. Embryos were collected from the cross *HSptc**F11*/+; *ptc*^{H84}/+ X *ptc*^{G12}/*en-lacZ*; amongst the progeny of this cross, *ptc*^{G12} heterozygous embryos also carry the *en-lacZ* gene¹⁹ and can therefore easily be distinguished from their hemizygous (*ptc*^{H84}/*ptc*^{G12}) sibs by visualizing their patterns of *lacZ* expression. Embryos heat-shocked for 30 min at stage 8/9 were fixed 4 h later and hybridized with a *lacZ* probe. Of 172 embryos identified as *ptc* mutants, 55 (70% of those expected to carry the *HSptc* construct) showed a completely normal pattern of *ptc* expression (*h*), in contrast to the ectopic expression of *ptc* in their non-rescued (*n*=90) sibs (*g*). The remaining embryos, classified as partially rescued, showed variable ectopic *ptc* expression in one or two segments (not shown).

METHODS. A nearly full-length *ptc* cDNA clone was assembled from partial cDNAs (described in ref. 13): the *Eco*R1/*Sna*B1 fragment from the clone pc2 was ligated with *Eco*R1/*Sna*B1-digested pc7 plasmid. A *Dra*I fragment containing the entire *ptc* open reading frame was purified from the resulting plasmid and ligated with *Hpa*I-digested transformation vector pCaSpeRhs (ref. 20). The recombinant plasmid pHsptc3, containing the *ptc* open reading frame in the correct orientation relative to the heatshock promoter, was selected after restriction-enzyme analysis of miniprep DNA from trans-



formed colonies and used to transform *Drosophila* embryos by standard microinjection procedures. The sequence of the *ptc* cDNA used here is deposited in the EMBL Data Base under the accession number X17558. For heat-shock treatments, embryos were collected from appropriate parents over 2-h intervals, transferred to thin layers of 1% agarose on glass microscope slides and incubated in a plastic Petri dish floating in a water bath at 37 °C for 30-min intervals. After heat treatment, embryos were returned to 25 °C (ref. 8) prior to fixation for *in situ* hybridization or antibody staining as described in the legend to Figure 1. Embryos were hybridized with DIG-labelled single-stranded RNA probes following a modification (M. Klingner and P. Gergen, personal communication) of the protocol described in ref. 21.

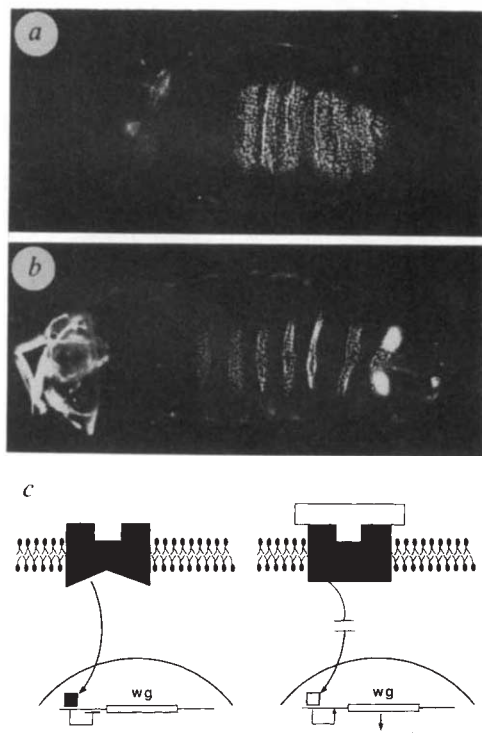


FIG. 3 The interaction between *ptc* and *hh*. *a* Dark-field image of the ventral cuticle of a first instar larva homozygous *hh*^U. These are easily identified by their reduced size and the absence of naked cuticle in each segment. *b*, By contrast, the *ptc*^{G12}; *hh*^U double-mutant embryos are significantly larger and show a phenotype similar to *ptc*. The double mutants could be unequivocally distinguished from their *ptc* sibs by the presence of the *Ubx*¹ mutation on the *hh* chromosome which results in a transformation of the first abdominal segment to mesothorax. This is manifest in the absence of thick denticles in the first abdominal segment and the presence of an additional partial Keilin's organ²². Some of the progeny of the same parents were fixed after eight hours of development and hybridized with *ptc* and *wg* probes. As *ptc*^{G12} is a deficiency of the entire *ptc* gene¹³, homozygous embryos can be distinguished by the absence of *ptc* expression. The pattern of *wg* expression seen amongst *ptc*⁻ embryos was identical, even though a quarter of these must be *hh*⁻. Thus absence of *ptc* removes the dependence of *wg* transcription on *hh* expression, resulting in the observed suppression of the *hh* phenotype. *c*, Hypothetical mechanism for the control of *wg* transcription by *ptc* and *hh*. In cells competent to express *wg*, transcription is blocked by *ptc* protein (filled shape) in the membrane, which could activate a transcriptional repressor for example (filled square). When *ptc* protein binds *hh* protein (open shape), it is inactivated, leading in turn to the inactivation of the transcriptional repressor molecule (open square) and hence to the activation of *wg* transcription.

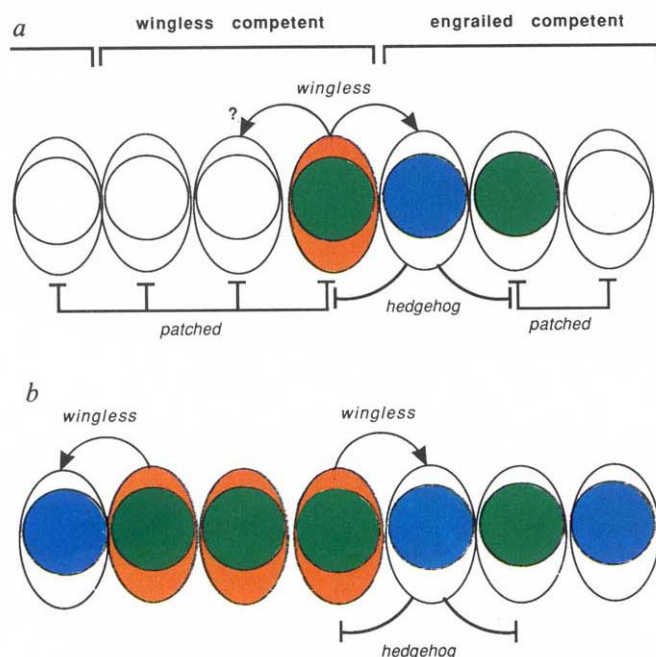


FIG. 4 A model for cellular interactions after gastrulation. To rationalize the differential response of cells to the local signals encoded by *wg* and *hh*, we propose that cells already have a latent capacity to express one or other of the *en* or *wg* genes; this predisposition probably depends on the prior activity of the various pair-rule genes expressed in the blastoderm embryo, although at present we have no explanation as to how cells retain a 'memory' of these activities. For simplicity, we represent a single-segment repeat by six cells; note that the cell on the extreme left is equivalent to that on the extreme right. The patterns of gene expression indicated by the different colours represent stable states of transcription in the stage-11 embryo that depend on the prior interactions indicated by the black lines. Orange cytoplasm represents *wg* expression; green nuclei, *ptc* transcription; and blue nuclei, *en* expression. In the wild-type embryo (a) *ptc* protein is initially expressed in all cells except for those expressing *en*; this expression serves to repress transcription both of *wg* and of *ptc* itself, unless the activity of the protein is antagonized by *hh*. This results in the maintenance of *ptc* transcription in cells on either side of the *en* cell, but of only *wg* in cells anterior to the *en* cell, because only these are predisposed to express *wg*. Similarly, the *wg* signal maintains *en* expression, but only in cells posterior to the *wg* cell as these alone are predisposed to express *en*. b, In the absence of *ptc* activity, all cells, except those expressing *en*, now transcribe *ptc*, whereas all cells with the appropriate competence express *wg*. This results in the juxtaposition of cells predisposed to express *en* with *wg*-expressing cells, resulting in the ectopic induction of *en*.

as in the case of *wg*, cells at different locations within each parasegment have differing capacities to respond to the *hh* signal. Interestingly, in embryos mutant for both *ptc* and another segment polarity gene *naked* (*nkd*), all of the cells in each parasegment express either *en* or *wg* (our unpublished observations). This suggests that cells are somehow endowed with the potential to express one or other gene, potentials that are suppressed unless cells receive the appropriate signals in the form of *wg* or *hh* proteins. □

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Unrestricted expression of the *Drosophila* gene *patched* allows a normal segment polarity

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IN the *Drosophila* embryo, mutations in the segment polarity gene *patched* (*ptc*) cause the replacement of the middle region of each segment by a mirror-image duplication of the remaining structures, including the parasegmental border¹⁻³. This gene, which encodes a transmembrane protein, is initially expressed in a generalized way at blastoderm, but later stops being transcribed in cells expressing the *engrailed* gene, and even later in cells in the middle of the parasegment²⁻⁴. The genes *engrailed* (*en*) and *wingless* (*wg*) are also segment-polarity genes, and they are expressed in adjacent stripes flanking the parasegment borders in the embryo⁵; in *ptc*

mutants *wg* expression extends anteriorly and an ectopic stripe of *en* expression is induced^{6,7}. The suggestion has been made that *ptc* must be transcribed in a specific subset of cells to prevent *en* expression anterior to the *wg*-expressing stripe⁴. Here we report that unrestricted expression of *ptc* from a heat-shock promoter has no adverse effect on development of *Drosophila* embryos. The heat-shock construct can also rescue *ptc* mutants, restoring *wg* expression to its normal narrow stripe. The ectopic *en* stripe fails to appear, but the normal one remains unaffected. The results imply that, despite its localized requirement, the restricted expression of *ptc* does not itself allocate positional information.

To analyse the spatial requirements for *ptc*, we have constructed transgenic flies (*HSptc*) in which *ptc* expression is directed by the heat shock protein hsp70 promoter (see Fig. 1 legend). Heat-shock pulses of 90 min induce high levels of *ptc* product at any stage of development, as detected by whole-mount immunohistochemical staining using anti-*ptc* antibody. The distribution of the protein shortly after the heat shock differs, depending on the stage. Thus if the pulse is applied at blastoderm, the levels are uniform (Fig. 1c), but if applied to embryos older than stage 9, the *ptc* product appears in a heterogeneous pattern which differs from that of the wild type (Fig. 1b, d). Therefore, the heat induction overrides both the early repression of *ptc* in *en* cells and its late constriction to

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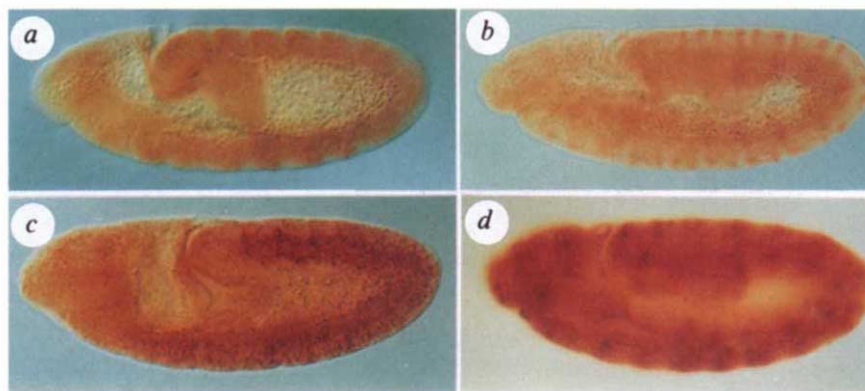


FIG. 3 Recovery of *wg* and *ptc* normal expression by *HSptc*. *a-c*, *In situ* hybridizations to *wg* RNA (blue signal) in embryos: *a*, *ptc*⁺; *b*, *ptc*^{IN}; *HSptc* non heat-shocked, and *c*, *ptc*^{IN}; *HSptc* heat-shocked at 36 °C for 90 min at germ band extension and fixed 90 min after the pulse. *d-f*, *In situ* hybridizations to *ptc* RNA (blue signal) in embryos: *d*, *ptc*⁺; (*e*), *ptc*^{IN}; *HSptc* non-heat-shocked, and *f*, *ptc*^{IN}; *HSptc* heat-shocked at 36 °C for 90 min at germ band extension and fixed 90 min after the pulse; *g* and *h*, *ptc*^{IN}; *HSptc* embryos heat-shocked at 36 °C for 90 min at germ band extension, fixed 30 min after the pulse and hybridized with *g*, *wg* or *h*, *ptc* probes.

METHODS. All embryos are from a stock *ptc*^{IN}/CyO, *hb-βgal*; *HSptc*, such that lack of brown signal in the head marks for *ptc*^{IN} homozygotes. The CyO, *hb-βgal* balancer was from G. Struhl. For protein/RNA double detection, embryos were first stained with anti-βgal (Cappel), then whole mount *in situ* hybridizations were performed according to ref 18. The *wg* probe¹⁹ was from P. Lawrence. Although the *ptc* probe labels both *HSptc* and endogenous *ptc* transcripts, the former decay rapidly and 30 min after the pulse only the latter are detectable. We have confirmed this in similar experiments using the allele *ptc*^{P78} (ref. 2), which produces no transcripts.

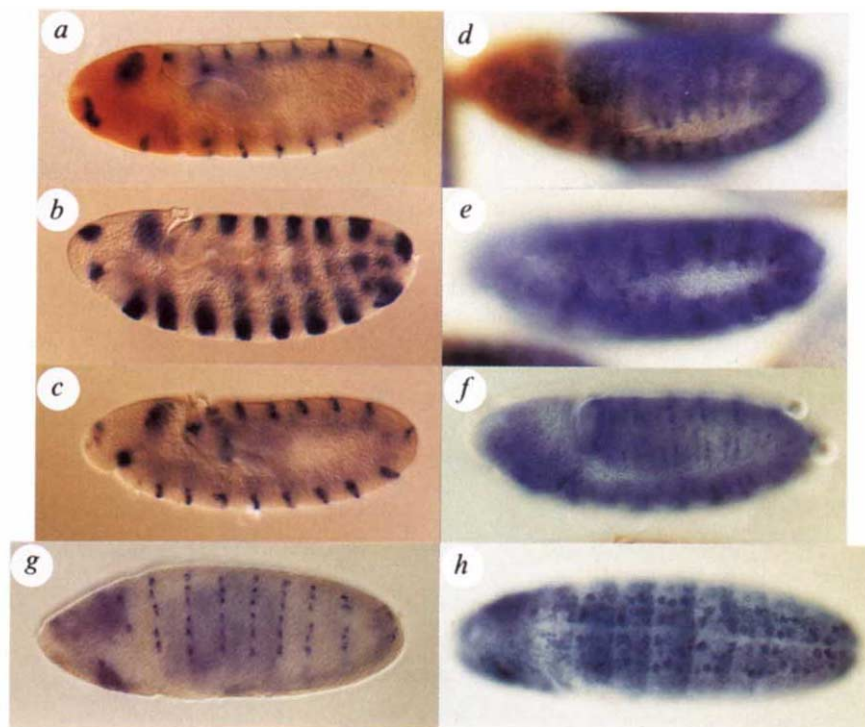


FIG. 4 Expression of *en* in *ptc*^{IN}; *HSptc* embryos. *a*, Non-heat-shocked embryos at stage 10, showing the ectopic *en* stripe; *b*, embryos at stage 11 heat-shocked at stage 8 for 90 min and fixed 60 min after the heat shock; *c*, embryos at stage 13 heat-shocked at stage 10 or 11 for 90 min and fixed 60 min after the heat shock.

METHODS. Immunostaining with anti-*invected* antibody (T. Kornberg) was by standard methods¹⁵.

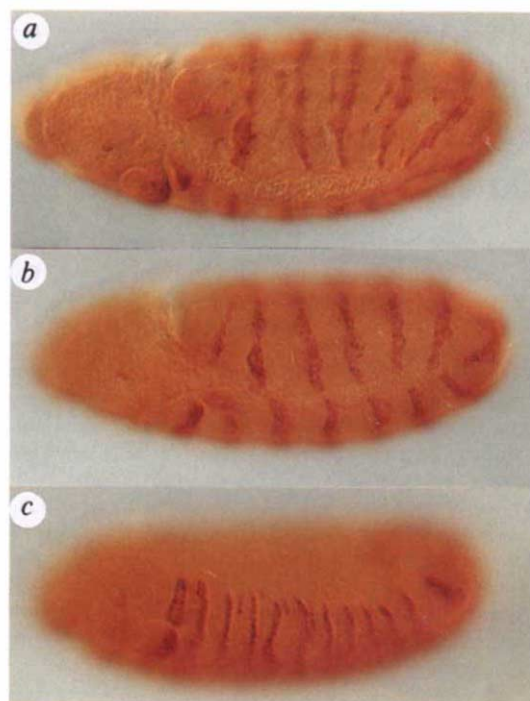


FIG. 1 Accumulation of *ptc* protein in *HSptc* embryos without (*a, b*) and with (*c, d*) heat shock. *a* and *b*, Anti-*ptc* antibody staining of *HSptc* embryos without heat-shock at stage 9 (*a*) and at stage 11 (*b*). The pattern of *ptc* protein is identical to the wild type and similar to that described for the RNA at those stages. Unstained stripes in *a* correspond to *en*-expressing cells. The two stripes labelled in each parasegment in *b* are contiguous to *en* cells. *c* and *d*, Whole-mount immunostaining with anti-*ptc* antibody of *HSptc* embryos heat-shocked at 36 °C for 90 min before (*c*) or during (*d*) germ-band elongation and fixed 20 min after the pulse. The embryo in *c* is in stage 9 and shows generalized expression of *ptc*. The embryo in *d* is in stage 11 and also shows generalized high levels of protein, although a superimposed pattern is observed which is not coincident with the wild type at this stage.

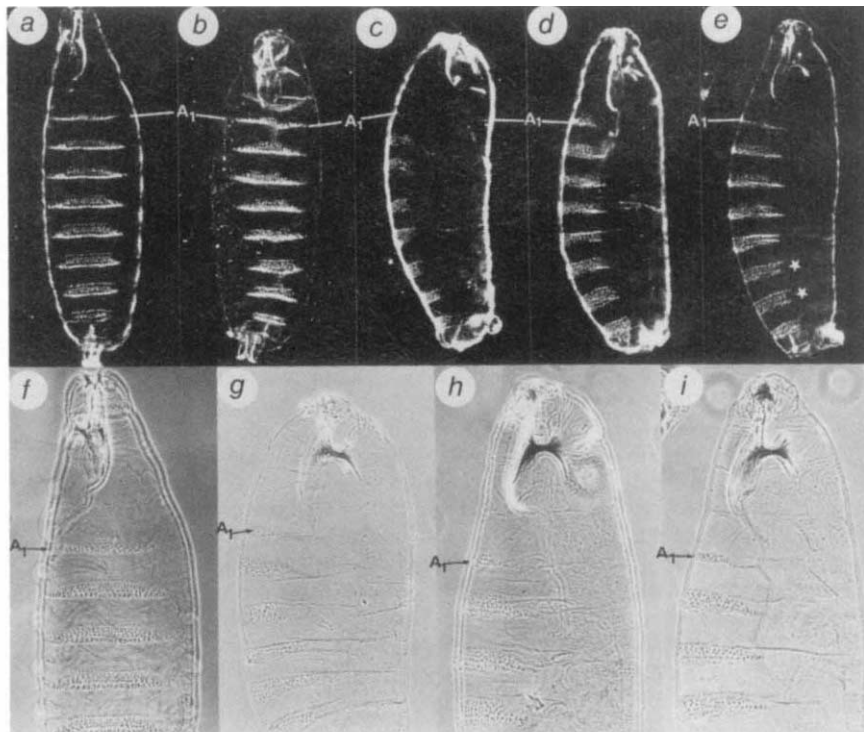
METHODS. Fly strains containing *ptc* complementary DNA coding sequences under the control of an inducible promoter (*hsp70* heat-shock promoter) were generated using P element-mediated germ line transformation¹². The transforming vector DNA was a modified Carnegie-20 P element vector containing a *hsp70* promoter and 3' transcription terminating sequences from the $\alpha 1$ tubulin gene¹³ in which we inserted from +481 to +4732 base pairs (bp) of the *ptc* cDNA². This cDNA contains the *ptc* coding sequences with only 270 bp of the 5' leader region and 80 bp of the 3' untranslated region. Several independently transformed lines were obtained. Here we have analysed one of these lines that contains a single P insertion on the third chromosome and is homozygous viable. We have also made other transformed lines with the same construction but containing a full length *ptc* cDNA; the behaviour of these lines is similar to the one described. To produce antibodies against the *ptc* protein, we cloned a *Bam*HI-*Pst*I fragment of the *ptc* cDNA (1,065 bp–1,789 bp)² into the *Eco*RI-*Bam*HI site of a pGEX-1 vector¹⁴ to produce a glutathione S-transferase fusion protein. This protein was excised from a polyacrylamide-SDS gel and collected by electroelution. Antiserum against *ptc* protein was raised by several subcutaneous injections with the fusion protein into young rats. Embryos were stained according to standard methods¹⁵.

two narrow stripes. The ectopic protein decreases rapidly and is hardly detectable 1 h after the pulse.

Surprisingly, heat-shocked embryos hatched as larvae without major cuticular anomalies. In a homozygous stock of *HSptc*, between 75 and 90% of the larvae hatched after 90 min of heat shock during germ-band extension with the normal segmentation pattern. Even multiple heat shocks at several developmental stages fail to produce alterations in segmental morphology and the larvae eclose as normal flies. Some *HSptc* transformant lines show, with low penetrance after stronger heat treatments, mirror-image duplications of the thoracic denticle belts and occasional extra denticles in the abdomen (Fig. 2*b*). Thus inappropriate expression of *ptc* outside its normal domain apparently does not cause any major effect on metameric pattern. One significant implication of this result is that *ptc* is unlikely to have a primordial role in the specification of positional fates within the segment.

To check whether *HSptc* can functionally replace the endogenous *ptc* gene, we examined the effect of *HSptc* in a *ptc*⁻ background. The *ptc*⁻ embryos die before hatching with defects in head involution, lack of thoracic belts and square-shaped, mirror-symmetrical abdominal belts. As shown in Fig. 2*e, h* and *i*, all three features are almost completely corrected in *ptc*⁻; *HSptc* larvae after 90 min of heat shock. A high proportion of the *ptc*⁻ embryos hatched under these conditions. Variable levels of normalization are observed for single pulses of 60 min at between 2.5 and 8 h of development, with a peak of maximum rescue at about 3.5–5.5 h. This is consistent with the period of requirement estimated for other segment-polarity genes^{8,9}. In the intermediate cases (Fig. 2*d*), the first structures to become rescued are the thoracic and the more anterior abdominal belts (see Fig. 2 legend). A similar gradation in the cuticular

FIG. 2 Cuticular phenotypes conferred by *HSptc* in presence or absence of the endogenous *ptc* gene. *a–e*, Dark field view of the ventral aspects of larvae; *f–i*, phase contrast view of higher magnifications of the head, thoracic and some abdominal segments of representative examples. *a*, Wild-type larva; *b*, a case of *HSptc* phenotype. Note the duplication of the thoracic belts and some extra denticles in the abdomen. This kind of phenotype appears in up to 20% of the embryos that are heat-shocked at 36 °C for 2 h at gastrulation; *c*, *ptc*^{IN} (ref. 16) larva showing the typical *ptc* null phenotype; *d* and *e*, two examples of *ptc*⁻; *HSptc* larva heat-shocked for 90 min at germ-band elongation (between stage 6 and stage 9 of embryogenesis) with lesser (*d*) or larger (*e*) phenotypic correction. Comparison with *c* shows that head involution is rescued, thoracic belts reappear, and abdominal belts tend to recover their wild-type shape, mainly in the anterior part of the abdomen. The A6 and A7 abdominal denticle belts (white stars) are never totally corrected. A similar gradation in the abdominal phenotype is observed in *ptc*^{IS} larvae raised at 18 °C, the permissive temperature for this allele (*ptc*^{IF}; ref. 16). To identify unequivocally the rescued *ptc*-phenotype, we recombined the *Distal-less* mutation *Dll*^{MP} (ref. 17) with *ptc*^{IN} to make a *Dll*^{MP}*ptc*^{IN}; *HSptc* stock (*Dll* embryos lack maxillary and antennal sense organs¹⁷). Although the *Dll*^{MP} *ptc*^{IN}; *HSptc* embryos are lethal as a result of the *Dll* mutation, their *ptc*⁻ phenotype after heat shock is identical to the one described for the hatched *ptc*⁻ larvae in the *ptc*^{IN}; *HSptc* stock. *f* and *g*, Detailed view of the anterior part of a wild type larva (*f*) and of a *ptc* null phenotype (*g*); *h* and *i* show two examples of *ptc*^{IN}; *HSptc* larvae



after 90 min of heat shock at germ band elongation. Note that the thoracic denticle belts are not totally corrected.

phenotype is observed in a *ptc* weak mutant (*ptc*^{IF}). Thus, the *HSptc* construct provides a functional *ptc* product which is able to fulfil the *ptc*⁺ role, despite being expressed unrestrictedly.

We have looked at the expression of *wg* and *en* to examine the basis for the rescue. In *ptc*⁻ (or non-heat shocked *ptc*⁻; *HSptc*) embryos, the expression of *wg* spreads forwards from its normal domain (Fig. 3b) and later an ectopic *en* stripe appears in front of the extended *wg* domain (Fig. 4a). In heat-induced *ptc*⁻; *HSptc* embryos, 30 min after the end of the pulse, *wg* transcripts disappear from the epidermis and remain in a group of neuroblasts (Fig. 3g). This phenomenon also occurs in *ptc*⁺; *HSptc* embryos, and suggests that the activity of *wg* may be differentially controlled in epidermis and nervous system. In control embryos heat-shocked in parallel we do not observe the specific repression. One hour after the pulse, the *wg* signal starts to reappear in the epidermis, but now with its typical narrow wild-type pattern (Fig. 3c). The ectopic *en* stripe does not appear at any time after the pulse however. Even 3 h after heat shock, when *wg* expression has spread anteriorly again, the ectopic *en* stripe is not formed, although the normal *en* stripe remains unaffected at any time after heat shock (Fig. 4b).

It has been suggested that the *ptc* product is required to prevent the *en* gene from being activated anterior to the *wg*-expressing cells⁴ and that the absence of *ptc* posterior to the *wg*-expressing cells allows the maintenance of *en* there⁴. Nevertheless, we have shown here that *HSptc* does not seem to affect the normal *en* stripe, even though the *ptc* product is present at high levels in *en*-expressing cells. Furthermore, when the heat-shock pulse is performed later, it fails to inhibit even the ectopic *en* stripe (Fig. 4c).

The establishment of the late pattern of *ptc* transcription depends on selective repression mediated by *ptc* product⁴. Thus, in some *ptc* alleles the *ptc* promoter fails to restrict its own activity to two narrow stripes flanking *en*-expressing cells. We studied the pattern of transcription of the endogenous *ptc* gene at different times after heat shock. Thirty minutes after the pulse, *ptc* expression is reduced in the epidermis, remaining stronger in the neuroblasts (Fig. 3h). One hour later, the activity of the endogenous *ptc* promoter has reappeared in its restricted wild-type location (Fig. 3f). Thus *wg* and endogenous *ptc* expression are restricted to the vicinity of the parasegmental border at this time. This pattern of expression is the same as in wild type and it seems to be a relatively stable situation 1 h after heat shock. It is possible that the expression of these two genes is maintained by the neighbouring *en*-transcribing cells, because *wg* and *ptc* expression decay in *en* mutants^{4,6}. Initially, however, *HSptc* causes *wg* to be repressed even in that position (Fig. 3g). This is probably a consequence of abnormally high levels of the heat-induced protein, as *ptc* product is present in *wg*-transcribing cells in normal development. It suggests that this excess of *ptc* is counteracting the stimulative effect of the neighbour *en*-expressing cells. Equally, it is also likely that persistent abnormally high levels of *ptc* protein cause pattern defects as a result of *wg* repression. However, as the half life of *ptc* protein is less than 1 h, this situation cannot be maintained by a continuous heat shock without killing the embryo.

Segmental patterning is conceivably based on the modulation of some general cellular phenomena by localized factors. Candidates for these local factors are the products of spatially restricted segment-polarity genes^{10,11}. It has been suggested that spatially restricted expression of segment polarity genes defines specific regions within the segment, and that these regions then interact to generate the segmental pattern⁶. In this model *ptc* must function in only a subset of the cells to specify a region of the

segment^{2-4,6,7}. We have shown, however, that universal expression of *ptc* does not irreversibly disturb a normal segment pattern, although it can rescue *ptc*⁻ mutants. Our results suggest that the expression of *ptc* does not by itself allocate a particular positional value. The localized requirement for *ptc* could mean that its product is mediating transmission of information which is due to another localized factor. □

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Scientific visualization: practices and promises

L. Eric Greenwade

Scientific visualization is one of the most influential forces in the way computers are used in research. The components, users and benefits of scientific visualization systems are described.

THE term scientific visualization was created in October 1986 by a panel sponsored by the National Science Foundation (NSF) to describe the application of graphics and imaging techniques to computational science. This Panel on graphics, image processing and workstations was to provide input to the NSF Division of Advanced Scientific Computing (DASC) on the relative importance of hardware and software for graphics and image processing at research institutions involved with advanced scientific computing. With the establishment of NSF-funded supercomputing centres, scientific users had quickly surpassed the ability of the then available tools for data analysis and display¹. More and more, the demands placed on visualization forced it to encompass other disciplines, such as computational geometry and data base theory², as well as a more thorough understanding of the human perceptual system³.

System set-up

Like all computing environments, a visualization system has a hardware and software component. The first usually consists of a scientific/engineering workstation with a large colour display. Because of the computing intensity of these problems, additional hardware is often provided to speed up the image generation process, such as large memories, multiple central processors, high-speed, large-capacity disks and special purpose hardware dedicated to the rapid display of lines and polygons on the display surface. Most computers, from personal to supercomputers, can perform in this capacity to some degree⁴. Specialized hardware performs the best, but usually at a considerable additional expense.

The software is the key to a successful, that is, useful visualization system. In many cases, the demands of the software drive the development and implementation of the hardware component and must be capable of importing data into the system in such a manner that further processing will have all the necessary parameters available. This includes such details as the number of dimensions, the extent of each dimension and data type or types. Frequently, the analysis of interest will involve the correlation between

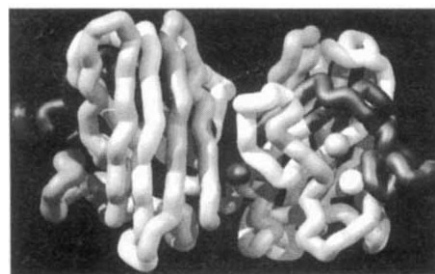
several quantities of different types, such as scalars, vectors and tensors. Ideally, the software must be able to transform the data to any new form that the user may request. These transformations need to occur rapidly, so that the analyst may concentrate on the investigative process. Providing this level of flexibility, in a methodology that is easily mastered, is a prime goal of a visualization system.

In this process, the data must often be filtered so that the relevant portion is being manipulated. A mapping process will then take the data to the appropriate domain where it will be converted to geometric primitives. These primitives may be anything from zero-dimensional points to three-dimensional volume elements or voxels. With each type of geometric primitive there is an associated set of attributes that help provide distinguishing characteristics to the various elements. At this point, the rendering process produces an image that can then be analysed for correctness and content⁵.

In this fashion, a researcher may access data sets that quickly are becoming measured in terms of megabytes and gigabytes. These data are then converted to a multi-dimensional image that can be interactively manipulated, allowing the complexities and intricacies to be observed and analysed. By manipulating the way in which the image is generated (for example, data classification, colour, texture, light sources and opacity), many different views of the same data may be created in quick succession and, thus, one can achieve greater intuition into the significance of the data. As has been most aptly put, "The purpose of computing is insight, not numbers"⁶.

Areas of application

A typical example involves the analysis of a molecule that protects cells from one of the by-products of metabolism. The human CuZn superoxide dismutase dimer is a metallo-enzyme that originated before aerobic life. In the figure, solid tubes that have been colour-coded by exon are used to trace the α carbon backbone of the dimer. This mapping indicates which exons are involved in dimer contacts and which map to sites on the surface of the protein⁷. The information was used to locate a buried junction that



Human CuZn superoxide dismutase. (Courtesy of the Scripps Research Institute, La Jolla, California.)

is believed to be a relic of the ancestral gene encoding. The original image was rendered using 10,000 spheres and photographed off of a high-resolution, 24-bit display. Insight of this kind would be difficult to obtain from the more traditional model-building method of displaying protein structures.

With most areas of our society embracing the computer, few will go untouched by scientific visualization. While the traditional areas such as medical imaging and computational fluid dynamics will continue to expand their utilization of visualization, new areas of application are being discovered daily. Such diverse fields as theoretical mathematics, battlefield simulation, architectural and mechanical design are capable of making accelerated advances using these new tools⁸. Equally important, the commercial and exploratory arts stand ready to enter new realms due to the power and versatility of visualization systems. Some areas of application have included human figure⁹ and facial¹⁰ animation. Many have observed the special effects used in the film industry. To apply natural appearing motion, collaboration with specialists in other fields, such as physiology and biomechanics, has become an integral part of the animation process.

In addition to producing higher quality and faster versions of existing hardware, there will be inroads made into new areas of increased productivity. For output, computer-generated holograms and three-dimensional stereo lithography hold many promises for future development. We are already experiencing the benefits of virtual reality by being able perceptually to enter the computer models with the use of stereo video displays. The use of audio is also being investigated as an additional channel for bring-

ing information to the user.

Another area of expansion is likely to involve the distribution of the visualization process. Already, numerous facilities have a large number of computers that could, through software and high-speed networks, be applied to a single visualization task. Many of the massively parallel computers will map easily onto visualization problems because of the inherent fine-grained parallelism in many of these tasks. There is also the opportunity for the creation of distributed laboratories in which many researchers, at geographically dispersed locations, may collectively and simultaneously manipulate, analyse and display the same data set.

Looking ahead

The directions for future development of visualization systems will be limited only by the imagination and resourcefulness of the users and the ability of vendors to provide timely solutions. While the speed of the hardware and the applicability of the software will always lag behind the need for these capabilities, the market has become sufficiently commercial for it to be cost effective for someone to provide a solution. Most new work occurs in government and/or academic research institutions, but the commercial market will be quick to apply advances to the much larger and less specific communities.

The next several years will find very few areas of endeavour where scientific visualization does not play a significant role. Already users are reaping the benefits in applications as diverse as medical imaging, economics, computational fluid dynamics, product design and commercial advertising. In spite of their relatively high cost, these visualization systems are providing advantages in the increasing competitive world. □

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Bulletin board

An integrated set of six new polymer modelling programmes, a statistical computing language and system for scientific data analysis, statistics, graphics and mathematical computing, and a new range of desktop visualization systems — new product ideas for computer buffs.

SPYGLASS has entered the UNIX arena with a version of Transform, the company's **data visualization software** for visualizing large data sets, for Silicon Graphics' entire IRIS 4D product line (*Reader Service No. 101*). Transform for Silicon Graphics' workstations, which will be available next month, uses the Motif windowing system and allows users with multi-dimensional data sets to create images from their data using advanced visualization techniques simply by pointing and clicking, Spyglass says. The software is best known for its colour raster imaging technology, which converts a range of data values to a colour spectrum, thus presenting a vivid image of the data. Users can explore and highlight detail by altering the colour palette. In addition, data can visually be seen as polar images, line graphs of rows or columns, or as contour, surface and vector plots. Two-dimensional arrays also can visually be seen as contour plots, where equal values are connected by lines. Transform allows the user to view the contour plot in black and white, colour or dashed-line format. The spacing of the contours can be adjusted or set automatically. Vector plots are created by selecting two data arrays that represent the X and Y components of the vector. Contour and vector plots can be overlaid onto another image and all images or graphs can be printed on Postscript printers. While Transform, which costs \$795 (US), works with two-dimensional data, multidimensional data sets can be viewed in slices. The program reads and converts most data formats, including 2D and 3D, Generic and HDF Image Files, TIFF, FITS, 2D ASCII Data, 2D ASCII Special and ASCII X-Y Column data.

Multichrom 2, the new **chromatography data system** from VG Data Systems, has been designed specifically to operate on the complete range of DEC VAX computers from the small, multi-user 3100 to the largest VAX 9000 (*Reader Service No. 102*). Multichrom's multi-user, multi-tasking capabilities allow each VAX system to support the individual activities of many Multichrom users simultaneously. Raw data are acquired from instruments by the VG Chromatography Server and transmitted as digitized data to Multichrom using Ethernet. Each user then processes chromatography data using the same algorithms and data analysis, thus facilitating the com-

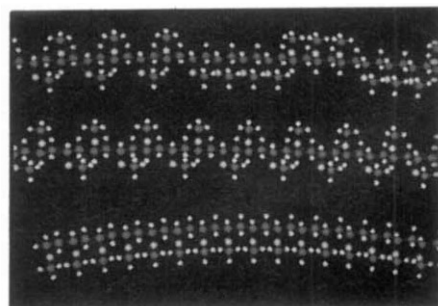


VG's chromatography data system.

parison of data, VG says. Operation is via an intuitive menu system, or by direct entry of command mnemonics. Other features of Multichrom 2 include full-screen browsing of files for selection or retrieval, integrated mouse control for the manipulation of chromatograms, and 'hot-key' menus throughout the system. Results can be reported and plotted on to a wide range of output devices. Plots, reports and raw data can be exported to a variety of other packages such as RS/1 and Lotus 1-2-3, and support is provided for the Digital Document Interchange Format (DDIF).

Molecules by design

Biosym, a developer of computer-aided molecular design systems, has introduced an integrated suite of six advanced **polymer modelling software products** that are accessed through Insight II — the company's three-dimensional (3-D) graphical molecular modelling interface (*Reader Service No. 103*). The products offer researchers access to a broad selection of scientific methods and theory, including atomistic methods, lattice theory, thermodynamics, empirical and semi-empirical methods and statistical mechanics. Polymerizer is an interactive program designed to build quickly 3-D



Polyvinylmethyl ether: ball-and-stick model by Biosym.

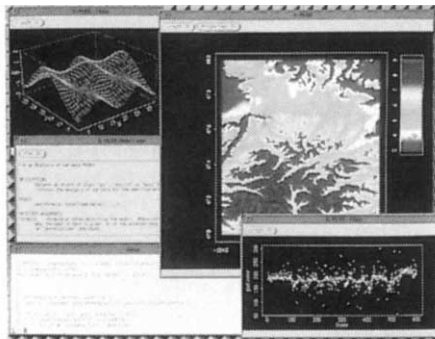
polymer models that are an accurate representation of real molecules. QSPR provides rapid empirical methods for making approximate predictions of a broad range of properties, Biosym says. Users can perform high-speed calculations of the statistical properties of single polymer chains, including molecular dimensions, chain flexibility, and optical properties in ideal solutions or in the bulk polymer state, with the RIS module. Amorphous Cell can be used to analyse dense amorphous polymer systems and Interphases calculates order parameters, statistical layer weights, configuration statistics, and solute distributions for monolayers and bilayers within interphases. Phase Diagram, which completes the line up, enables the researcher to explore ranges of temperature and composition for polymer blends and to determine optimum conditions for specific applications. Versions for IBM workstations will be available in December.

Cobra, a system for the **automated conformational analysis** and the generation of three-dimensional (3-D) structures from Oxford Molecular, is now available to users of Silicon Graphics' workstations (*Reader Service No. 104*). It is also available on other UNIX platforms, such as the Series 700 from Hewlett Packard and the Sun SPARCstations, as well as Digital Equipment's VAX/VMS systems. Cobra provides a means of rapidly searching the conformational space of molecules to identify minimum energy conformations, and should provide a useful tool for generating 3-D data bases. Input for Cobra is a two-dimensional representation of the molecule, in which the atom types, connectivity and, optionally, stereochemistry are defined. This information can be provided as a SMILES string (a chemical notation system), or by several 3-D file formats. The first stage of conformational analysis is the recognition of conformational units in the input structure. Conformational templates from Cobra's knowledge base are assigned to each unit and the templates are then joined to form complete conformations. Any resultant strain in these conformations can be resolved if necessary. Cobra can also perform combined configurational/conformational searching. Centres with ambiguous stereochemistry are identified and, if requested, the conformational search can be carried out over all configurations, for chiral centres and *cis/trans* double bonds, the company says. Cobra is available as a standalone package with a keyword-driven interface, or interfaced to the PIMMS graphics package.

Aids for data analysis

With over 1,000 built-in functions, S-PLUS version 3.0 from Statistical

Sciences offers an **interactive computing environment for graphics, data analysis, statistics and mathematical computing** (*Reader Service No. 105*). S-PLUS is an enhanced and commercially available version of the UNIX-based 'New S' interactive statistical programming language and system developed by res-



S-PLUS Version 3.0.

earchers at AT & T's Bell Laboratories. The S-PLUS programming environment is designed to offer the user complete flexibility in tailoring statistical and graphical analysis to any problem. Using the program's object-oriented language, users can write functions and/or interface to C and FORTRAN routines to create custom solutions to scientific data analysis and statistical problems. Publication-quality graphics can be output through Hewlett Packard's LaserJet and Postscript-based printers. Statistical Sciences offers a 30-day free evaluation period for S-PLUS, which is available on most UNIX systems as well as DOS computers.

Version 5.0 of SYSTAT, a **statistical analysis, graphics and data management software** package by a company of the same name, is now available for Digital Equipment's VAX/VMS computer systems (*Reader Service No. 106*). The VAX version of SYSTAT 5.0 offers dynamic colour graphics. In addition to basic graphics such as pie charts, bar graphs and plots, the new version offers high-resolution graphics for visualization such as three-dimensional rotation, maps (USA state boundaries and world map files are available), global mapping in true perspective with contouring, polar and spherical coordinates, triangular coordinate plots, contour plots, nonlinear scatterproof smoothing, Chernoff's faces, kernel density estimation plots and Voronoi tessellations. Virtual memory support permits analysis of very large data sets on the VAX, with enhanced support for up to 1,024 variables and an unlimited number of cases in each data file. Some of the statistical procedures that can be performed using SYSTAT 5.0 include descriptive statistics, frequency distributions, t-tests, nonparametric statistics, discriminant analysis, correlations,

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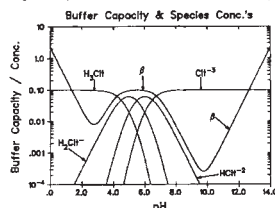
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simple linear and multiple linear regression, times series, forecasting and exponential smoothing, common factor analysis, nonlinear standard errors, ANOVA, ANOCOVA and MANOVA. The macro command processor provides VAX users with extensive screen handling capabilities, string format control and a powerful programming language, SYSTAT says. Repetitive tasks can be automated and facilities are provided for exporting data into documents.

Items of hardware

Stardent has introduced the VISTRA 800 Series, a new family of **desktop visualization systems** based on the Intel 40-MHz i860 microprocessor (*Reader Service No. 107*). By keeping the price around the £20,000 (UK) mark, Stardent hopes to target users in the research and scientific community whose applications could benefit from visualization but who previously had been unable to afford direct access to visualization systems. Three



The VISTRA 800.

models are available: the 800, 800e and the 800ex, each differing in their level of graphics performance. All are optimized to run AVS (Application Visualization System), Stardent's visualization software that allows users to bring the power of visualization to their application without graphics or user-interface programming. And, all offer integrated graphics and rendering capabilities. Virtual pixel maps technology allows the VISTRA 800 Series to process and display images simultaneously. This advanced technique renders images into virtual memory rather than directly to the frame buffer, Stardent says. This allows rendered images to be very large and allows flexible image and graphics processing irrespective of the size of the frame buffer and architecture. Other features include: four shading types (flat, smooth, normal and quadratic), single- and multi-pass true transparency, and two- and three-dimensional texture mapping. Prices for the VISTRA 800 Series range from £17,250 to £48,400 (UK). However, a typical configuration with 32-MB memory, 400-MB disk drive, a 19-inch colour

monitor, keyboard and mouse costs £22,950, £26,230 and £35,250 (UK) for the 800, 800e and 800ex, respectively.

Sun Microsystems has unveiled two new **desktop workstations**, the SPARCstation IPX and ELC, which are designed to provide some of the capabilities of Sun's high-end SPARCstation 2 but in a smaller, less expensive package (*Reader Service No. 108*). The SPARCstation ELC is aimed at users whose computing needs have outgrown the limitations of PCs and terminals, but who cannot afford high-end workstations. With a basic price of \$4,995 (US), Sun says the ELC offers multiple windows, a high-resolution display, fast response times, built-in networking and access to Sun's client-server environment. The SPARCstation IPX, which costs \$11,995 (US) (basic price), offers built-in GX accelerated graphics as a standard feature. This allows interactive manipulation of complex images, detailed charts and multiple windows in near real-time, Sun says. Both include SCSI, serial ports, and audio capabilities.

Maximum Storage has introduced a version of its **DUETTE optical disk subsystem** for Sun Microsystems' SPARC station family of workstations (*Reader Service No. 109*). The DUETTE optical disk subsystem is based around a 1 GByte multifunctional optical disk drive. Optical disks provide secure data storage and are a useful alternative to magnetic tapes or hard disks, the company says. With an average data transfer rate of 7.9 Mbits per second through its SCSI-2 interface, the drive provides fast, random access to large amounts of online



DUETTE — optical disk subsystem.

data. The multifunctional nature of the drive allows the user to select WORM (write once, read many) media where permanent data storage is required, or erasable media for data of a more temporary nature. In addition, DUETTE allows disks written by the PC to be read by the Sun SPARCstation and vice versa. Future versions of DUETTE, which will support VAX, Macintosh, PS/2 and other hosts, will expand further the range of computers capable of sharing data via the interchange of removable optical disks □